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L6: Entry 16 of 131

File: USPT

Jun 5, 2001

DOCUMENT-IDENTIFIER: US 6242194 B1

TITLE: Acid-inducible promoters for gene expression

DEPR:

The complete nucleotide sequence of the atp operon was determined (data not shown). Eight open reading frames (ORFs) with putative ribosome binding sites (RBSSs) and start codons were represented in the sequence of the contiguous clones. The gene order, atpBEFHAGDC, was identical to that observed in other bacteria (Santana et al., supra; Saraste et al. supra; Shibata et al., supra; Walker et al., supra). The start codon of each gene was designated by alignment of atp gene sequences of other bacteria and the position of possible RBSSs. TTG start codons appeared to be present for atpA and atpD, while the remaining six genes are proposed to begin with the ATG triplet. The eight gene products of the *L. acidophilus* atp operon were aligned with the ATPase subunits from *E. coli*, *B. subtilis*, *En. hirae* and *S. mutans* using the alignment algorithm of Myers et al. (*J. Comput. Biol.* 3, 563 (1996)) with a weight matrix of PAM250 (Table 1). The deduced amino acid sequences of the *L. acidophilus* ATPase subunits showed homology with those of other bacteria. The greatest homology was observed in the .alpha.,.gamma., and .beta. subunits, comprising the cytoplasmic domain (F.sub.1) of the ATPase. Less homology was evident for the a, b, and c subunits of the membrane-bound domain. The .delta. subunit of the F.sub.1 domain showed the greatest variability of all the subunits. Of the bacteria included in the comparison, the subunits of the *E. coli* ATPase exhibited the least homology with the corresponding products from *L. acidophilus*. The a, b, and c subunits of the F.sub.0 sector from *L. acidophilus* generally appeared most similar to the corresponding protein of *B. subtilis*, while the .alpha.,.gamma., and .beta. subunits of the membrane bound domain shared the greatest homology with *S. mutans* and *En. hirae*.

DETL:

TABLE 1 Similarities between the H.sup.+ -ATPase subunits from *L. acidophilus* and other bacteria. *B. subtilis* *E. coli* *En. hirae* *S. mutans* Gene Subunit % identity (% similarity) atpB a 34 (75) 17 (60) 36 (74) 37 (75) atpE c 52 (84) 37 (79) 43 (64) 33 (71) atpF b 32 (78) 19 (50) 37 (79) 35 (80) atpH .delta. 28 (75) 25 (74) 32 (73) 25 (72) atpA .alpha. 73 (93) 51 (86) 80 (96) 76 (95) atpG .gamma. 33 (75) 30 (72) 41 (80) 35 (78) atpD .beta. 61 (88) 58 (85) 73 (92) 71 (90) atpC .epsilon. 40 (79) 25 (72) 42 (70) 47 (81)

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TITLE: Acid-inducible promoters for gene expression

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TABLE 1 Similarities between the H.sup.+ -ATPase subunits from *L. acidophilus* and other bacteria. *B. subtilis* *E. coli* *En. hirae* *S. mutans* Gene Subunit % identity (% similarity) atpB a 34 (75) 17 (60) 36 (74) 37 (75) atpE c 52 (84) 37 (79) 43 (64) 33 (71) atpF b 32 (78) 19 (50) 37 (79) 35 (80) atpH .delta. 28 (75) 25 (74) 32 (73) 25 (72) atpA .alpha. 73 (93) 51 (86) 80 (96) 76 (95) atpG .gamma. 33 (75) 30 (72) 41 (80) 35 (78) atpD .beta. 61 (88) 58 (85) 73 (92) 71 (90) atpC .epsilon. 40 (79) 25 (72) 42 (70) 47 (81)



US006242194B1

(12) **United States Patent**
Kullen et al.

(10) Patent No.: US 6,242,194 B1
(45) Date of Patent: Jun. 5, 2001

(54) ACID-INDUCIBLE PROMOTERS FOR GENE EXPRESSION

5,994,077 11/1999 Valdivia et al. .

(75) Inventors: Martin J. Kullen; Todd R. Klaenhammer, both of Raleigh, NC (US)

(73) Assignee: North Carolina State University, Raleigh, NC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/637,968

(22) Filed: Aug. 11, 2000

Related U.S. Application Data

(63) Continuation of application No. 09/336,861, filed on Jun. 21, 1999, now abandoned.

(51) Int. Cl.⁷ C12Q 1/68; C12P 21/04; C12N 1/20; C12N 15/00; C07H 21/04

(52) U.S. Cl. 435/6; 435/252.3; 435/252.9; 435/320.1; 435/471; 536/23.1; 536/24.1

(58) Field of Search 435/6, 7.32, 69.1, 435/91.1, 91.31, 91.4, 91.5, 252.3, 252.9, 320.1, 476, 489, 853; 514/44; 536/23.1, 23.5, 24.1, 24.5, 25.3

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- | | | |
|-----------|---------|---------------------------|
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| 5,593,885 | 1/1997 | Klaenhamer et al. . |
| 5,618,723 | 4/1997 | Kaenhamer et al. . |
| 5,773,692 | 6/1998 | Johnson-Flanagan et al. . |
| 5,837,509 | 11/1998 | Israelsen et al. . |

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M.J. Kullen et al.; Use of Differential Display RT-PCR to Identify Conditionally Expressed Genes in *Lactobacillus Acidophilus*, Abstract, *American Society for Microbiology, ASM Conference on Small Genomes*, pp. 29-30 and cover page, Sep. 20-24, 1998 at Lake Arrowhead, California.

O'Sullivan et al.; Relationship Between Acid Tolerance, Cytoplasmic pH, and ATP and H⁺-ATPase Levels in Chemostat Cultures of *Lactococcus Lactis*, *Applied and Environmental Microbiology*, 65(6):2287-2293 (Jun. 1999). Collins et al.; Selection of Probiotic Strains for Human Applications, *Int. Dairy Journal*, 8:487-490 (1998).

Mary Ellen Sanders; Overview of Functional Foods: Emphasis on Probiotic Bacteria, *Int. Dairy Journal*, 8:341-347 (1998).

Madsen et al.; Molecular Characterization of the pH-Inducible and Growth Phase-Dependent Promoter P170 of *Lactococcus Lactis*, *Molecular Microbiology*, 32(1):75-87 (1999).

Primary Examiner—John L. LeGuyader

Assistant Examiner—Jane Zara

(74) Attorney, Agent, or Firm—Myers Bigel Sibley & Sajovec

(57) ABSTRACT

An isolated polynucleotide encoding an acid-inducible, or acid-responsive, promoter element includes the F_iF_o-ATPase promoter of *Lactobacillus acidophilus* DNA that hybridizes thereto and encodes an acid-inducible promoter. Recombinant molecules comprising the promoter operatively associated with a DNA of interest, along with vectors and host cells containing the same, are also disclosed. Methods of upregulating the transcription of a DNA of interest in a host cell with such promoters are also disclosed.

22 Claims, 7 Drawing Sheets

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USPT	(1 and gene\$) not 2	154	<u>L3</u>
USPT	(1 and gene\$) not 2	217	<u>L4</u>
USPT	gene or genetic or genetically or recombinant or orf or codon or vector coli	81343	<u>L5</u>
USPT	5 and 1	21	<u>L6</u>
USPT	atpg and atpd	1	<u>L7</u>
USPT	atpg and atpa	1	<u>L8</u>
USPT	atp near3 synthase	125	<u>L9</u>
USPT	9 and (gram or prokaryote or procaryote or prokaryotic or procaryotic or bacteri\$ or microorganism or micro-organism)	111	<u>L10</u>

Mutations in the mitochondrial ATP synthase gamma subunit suppress a slow-growth phenotype of ym_{E1} yeast lacking mitochondrial DNA.

Weber ER; Rooks RS; Shafer KS; Chase JW; Thorsness PE

Department of Molecular Biology, University of Wyoming, Laramie
82071-3944, USA.

Genetics (UNITED STATES) Jun 1995, 140 (2) p435-42, ISSN 0016-6731
Journal Code: FNH

Contract/Grant No.: GM-47390, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

In *Saccharomyces cerevisiae*, **inactivation** of the nuclear gene YME1 causes several phenotypes associated with impairment of mitochondrial function. In addition to deficiencies in mitochondrial compartment integrity and respiratory growth, ym_{E1} mutants grow extremely slowly in the absence of mitochondrial DNA. We have identified two genetic loci that, when mutated, act as dominant suppressors of the slow-growth phenotype of ym_{E1} strains lacking mitochondrial DNA. These mutations only suppressed the slow-growth phenotype of ym_{E1} strains lacking mitochondrial DNA and had no effect on other phenotypes associated with ym_{E1} mutations. One allele of one linkage group had a collateral respiratory deficient phenotype that allowed the isolation of the wild-type gene. This suppressing mutation was in ATP3, a gene that encodes the **gamma** subunit of the mitochondrial ATP synthase. Recovery of two of the suppressing ATP3 alleles and subsequent sequence analysis placed the suppressing mutations at strictly conserved residues near the C terminus of Atp3p. **Deletion** of the ATP3 genomic locus resulted in an inability to utilize nonfermentable carbon sources. **atp3 deletion** strains lacking mitochondrial DNA grew slowly on glucose media but were not as compromised for growth as ym_{E1} yeast lacking mitochondrial DNA.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *DNA, Mitochondrial--genetics--GE; *H(+)--Transporting ATP Synthase--genetics--GE; *Saccharomyces cerevisiae--genetics--GE; *Suppression, Genetic; Amino Acid Sequence; Escherichia coli--genetics--GE; Molecular Sequence Data; Phenotype; **Plasmids**; Saccharomyces cerevisiae--enzymology --EN; Saccharomyces cerevisiae--growth and development--GD; Sequence Alignment

Molecular Sequence Databank No.: GENBANK/U08318

CAS Registry No.: 0 (DNA, Mitochondrial); 0 (Plasmids)

Enzyme No.: EC 3.6.1.34 (H(+)--Transporting ATP Synthase)

Gene Symbol: ist/GeneSymbol YME1

Record Date Created: 19960117

In vivo synthesis of ATPase complexes of Propionigenium modestum and Escherichia coli and analysis of their function.

Gerike U; Kaim G; Dimroth P

Mikrobiologisches Institut, Eidgenossische Technische Hochschule, Zurich, Switzerland.

European journal of biochemistry (GERMANY) Sep 1 1995, 232 (2)
p596-602, ISSN 0014-2956 Journal Code: EMZ

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Adonis

Expression studies of Propionigenium modestum ATPase genes in various combinations with Escherichia coli ATPase genes were performed in the unc **deletion** mutant strain E. coli DK8. **Plasmids** containing the whole unc operon from P. modestum were unable to complement the E. coli unc **deletion** mutant. Although all ATPase subunits were **expressed** from the **plasmids**, there was no detectable ATP hydrolysing activity, indicating that the F1 part was not functional. Transformants **expressing** an E. coli F1-P. modestum F0 hybrid exhibited considerable ATPase activities. Binding of the F1 part to the membrane was very weak, however, and the coupling between ATP hydrolysis and Na⁺ transport was impaired. After combining the genes for E. coli ATPase subunits alpha, beta, **gamma**, delta and epsilon and the hydrophilic part of subunit b with P. modestum ATPase subunits a and c and the hydrophobic part of subunit b on a **plasmid**, a non-functional hybrid ATPase was **expressed** in E. coli. The ATPase was only loosely bound to the membrane, from which it was solubilized with Triton X-100 and purified. Subunit b and a proteolytic degradation product were the only F0 subunits detectable in the purified enzyme. A stable F0 complex is thus not formed with the hybrid b subunit. The absence of a functional F0 complex was in accord with proton-conduction measurements with bacterial vesicles. The only functional Na⁽⁺⁾-translocating ATPase **expressed** in E. coli thus far consists of E. coli subunits alpha, beta, **gamma** and epsilon, and P. modestum subunits delta, a, b and c [Kaim, G. & Dimroth, P. (1993) Eur. J. Biochem. 218, 937-944]. During the cloning conducted in our present study, errors in the sequence entry into the EMBL data bank (accession no. X58461) for the P. modestum ATPase alpha and beta subunits became evident, which are corrected in this paper.

Descriptors: *Adenosinetriphosphatase--biosynthesis--BI; *Escherichia coli--enzymology--EN; *Gram-Negative Anaerobic Bacteria--enzymology--EN; Adenosinetriphosphatase--chemistry--CH; Adenosinetriphosphatase--genetics--GE; Amino Acid Sequence; Base Sequence; Cloning, Molecular; DNA, Bacterial--genetics--GE; Escherichia coli--genetics--GE; Gene **Deletion**; Gene **Expression**; Genes, Bacterial; Genetic Complementation Test; Gram-Negative Anaerobic Bacteria--genetics--GE; H⁽⁺⁾-Transporting ATP Synthase--biosynthesis--BI; H⁽⁺⁾-Transporting ATP Synthase--chemistry--CH; H⁽⁺⁾-Transporting ATP Synthase--genetics--GE; Molecular Sequence Data; Operon; **Plasmids**--genetics--GE; Polymerase Chain Reaction; Protein Conformation; **Recombinant Proteins**--biosynthesis--BI; **Recombinant Proteins**--chemistry--CH; **Recombinant Proteins**--genetics--GE; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/J01594; GENBANK/X58461

CAS Registry No.: 0 (DNA, Bacterial); 0 (Plasmids); 0 (Recombinant Proteins)

Enzyme No.: EC 3.6.1.3 (Adenosinetriphosphatase); EC 3.6.1.34 (H⁽⁺⁾-Transporting ATP Synthase)

Record Date Created: 19951114

Alpha 3 beta 3 complex of thermophilic ATP synthase . Catalysis without the gamma -subunit.

Kagawa Y; Ohta S; Otawara-Hamamoto Y

Department of Biochemistry, Jichi Medical School, Tochigi-ken, Japan.

FEBS letters (NETHERLANDS) May 22 1989, 249 (1) p67-9, ISSN 0014-5793 Journal Code: EUH

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

A complex of the alpha- and beta-subunits of thermophilic ATP synthase showed about 25% of the ATPase activity of the alpha beta **gamma** complex. The alpha 3 beta 3 hexamer structure was analyzed by sedimentation (11.2 S) and gel filtration (310 kDa). Dilution of the alpha beta complex caused dissociation of the complex and rapid loss of ATPase activity which was restored by addition of the **gamma**-subunit. A previous method using urea for isolating the subunits resulted in an alpha beta complex with lower activity than that prepared by over- **expression** of the genes. The alpha beta-ATP complex was formed from the alpha beta complex, ADP and Pi in the presence of dimethyl sulfoxide.

Tags: Support, Non-U.S. Gov't

Descriptors: *Bacteria--enzymology--EN; *H(+)-Transporting ATP Synthase --isolation and purification--IP; Catalysis; Chromatography, Gel; H(+) -Transporting ATP Synthase--antagonists and inhibitors--AI; Molecular Structure; Structure-Activity Relationship

Enzyme No.: EC 3.6.1.34 (H(+)-Transporting ATP Synthase)

Record Date Created: 19890628

A respiratory-driven and an artificially driven ATP synthesis in mutants of *Vibrio parahaemolyticus* lacking H⁺-translocating ATPase .

Sakai Y; Moritani C; Tsuda M; Tsuchiya T

Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Japan.

Biochimica et biophysica acta (NETHERLANDS) Mar 23 1989, 973 (3)
p450-6, ISSN 0006-3002 Journal Code: A0W

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Mutants of *Vibrio parahaemolyticus* lacking the H⁺-translocating ATPase were isolated to evaluate both the role of this enzyme and the possibility of the involvement of other cation-translocating ATPase in the energy transduction in this organism. Dicyclohexylcarbodiimide-sensitive ATPase activity which represents the H⁺-translocating ATPase was not detected either in the membrane vesicles or in the cytosol of the mutants. Three major subunits, alpha, beta and **gamma**, of the H⁺-translocating ATPase were missing in the membranes of the mutants. Although ATP was synthesized in wild type **cells** when an artificial H⁺ gradient was imposed, little ATP was synthesized in the mutants. However, we observed a large ATP synthesis driven by the respiration not only in the wild type but also in the mutants. The respiratory-driven ATP synthesis in wild type was inhibited by an H⁺ conductor, carbonylcyanide m-chlorophenylhydrazone, by about 50%. On the other hand, the ATP synthesis in the mutants was not affected by the H⁺ conductor. Since this organism possesses a respiratory Na⁺ pump, Na⁺-coupled ATP synthesis might take place. In fact, we observed some ATP synthesis driven by an artificially imposed Na⁺ gradient both in the wild type and the mutant.

Tags: Support, Non-U.S. Gov't

Descriptors: *Adenosine Triphosphate--biosynthesis--BI; *H(+) -Transporting ATP Synthase--deficiency--DF; *Mutation; *Oxygen Consumption; *Vibrio parahaemolyticus--enzymology--EN; Carbonyl Cyanide m-Chlorophenyl Hydrazone ; H(+) -Transporting ATP Synthase--metabolism--ME; Oxidative Phosphorylation ; Sodium Chloride; Vibrio parahaemolyticus--genetics--GE; Vibrio parahaemolyticus--isolation and purification--IP

CAS Registry No.: 555-60-2 (Carbonyl Cyanide m-Chlorophenyl Hydrazone); 56-65-5 (Adenosine Triphosphate); 7647-14-5 (Sodium Chloride)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19890508

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USPT	1 and 2	1	<u>L3</u>
USPT	f1 same (atp or atpase or f1atpase or f1atp or atp\$)	51	<u>L4</u>

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USPT	1 and 2	1	<u>L3</u>
USPT	f1 same (atp or atpase or f1atpase or f1atp or atp\$)	51	<u>L4</u>

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USPT	(atp or atpase or f1atpase or f1atp or atp\$).clm.	672	<u>L2</u>
USPT	1 and 2	1	<u>L3</u>
USPT	f1 same (atp or atpase or f1atpase or f1atp or atp\$)	51	<u>L4</u>
USPT	(atp or atpase or f1atpase or f1atp or atp\$) same gamma	1923	<u>L5</u>
USPT	5 same (subunit or sub-unit)	131	<u>L6</u>

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USPT	(atp or atpase or f1atpase or f1atp or atp\$).clm.	672	<u>L2</u>
USPT	1 and 2	1	<u>L3</u>
USPT	f1 same (atp or atpase or f1atpase or f1atp or atp\$)	51	<u>L4</u>
USPT	(atp or atpase or f1atpase or f1atp or atp\$) same gamma	1923	<u>L5</u>
USPT	5 same (subunit or sub-unit)	131	<u>L6</u>

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L6: Entry 23 of 131

File: USPT

Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214591 B1

TITLE: Methods for producing L-valine and L-leucine

BSPR:

H_{sup.+} -ATPase is a membrane-binding enzyme with approximately 500,000 KD in molecular weight, in which 8 types of subunits complicatedly associate, and functions to pump H_{sup.+} outside of cytoplasm through changes in the free energy caused by ATP hydrolyzation and to synthesize ATP utilizing a H_{sup.+} -concentration gradient between the inside and outside of cytoplasmic membrane caused by intracellular respiration. Additionally, this enzyme is divided into an F0 fraction, which is localized on the inner membrane and exhibits H_{sup.+} -transport activity, and an F1 fraction, which is localized in the membrane surface and catalyzes the decomposition and synthesis of ATP, and the F0 is composed of 3 types of subunits a, b and c, while the F1 is composed of 5 types of subunits .alpha., .beta., .gamma., .delta., .epsilon.. A strain which has a mutation in any of these subunits can be used as a H_{sup.+} -ATPase-deficient strain. The mutation of the H_{sup.+} -ATPase deficiency may include the expression of a mutant subunit, and the non-expression of subunits comprising H_{sup.+} -ATPase by the mutation at a promoter site.

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L6: Entry 23 of 131

File: USPT

Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214591 B1

TITLE: Methods for producing L-valine and L-leucine

BSPR:

H_{sup.+} -ATPase is a membrane-binding enzyme with approximately 500,000 KD in molecular weight, in which 8 types of subunits complicatedly associate, and functions to pump H_{sup.+} outside of cytoplasm through changes in the free energy caused by ATP hydrolyzation and to synthesize ATP utilizing a H_{sup.+} -concentration gradient between the inside and outside of cytoplasmic membrane caused by intracellular respiration. Additionally, this enzyme is divided into an F0 fraction, which is localized on the inner membrane and exhibits H_{sup.+} -transport activity, and an F1 fraction, which is localized in the membrane surface and catalyzes the decomposition and synthesis of ATP, and the F0 is composed of 3 types of subunits a, b and c, while the F1 is composed of 5 types of subunits .alpha., .beta., .gamma., .delta., .epsilon.. A strain which has a mutation in any of these subunits can be used as a H_{sup.+} -ATPase-deficient strain. The mutation of the H_{sup.+} -ATPase deficiency may include the expression of a mutant subunit, and the non-expression of subunits comprising H_{sup.+} -ATPase by the mutation at a promoter site.

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L6: Entry 59 of 131

File: USPT

Aug 31, 1999

DOCUMENT-IDENTIFIER: US 5945505 A

TITLE: Human phospholemann-like protein

BSPR:

The sodium potassium ATPase (Na,K-ATPase) .gamma.-subunit, formerly known as the Na,K-ATPase proteolipid, is a small membrane protein that co-purifies with the .alpha.- and .beta.-subunits of Na,K-ATPase (Mercer R W et al (1993) J Cell Biol 121:579-586). The .gamma.-subunit is a small membrane protein consisting of 58 amino acids with a single transmembrane domain. This transmembrane domain is structurally related to the transmembrane domains of other PLM-like proteins. The .gamma.-subunit may act as a regulator of the ATP-dependent ion channel activity of Na,K-ATPase.

BSPR:

The present invention features a novel human PLM-like protein, hereinafter referred to as HPLP, having chemical and structural homology to PLM, Mat-8, CHIF, and Na,K-ATPase .gamma.-subunit. Accordingly, the invention features a substantially purified HPLP, encoded by amino acid sequence of SEQ ID NO:1, having structural characteristics of the family of PLM-like proteins.

DRPR:

FIG. 2 shows the amino acid sequence alignments among HPLP (SEQ ID NO:1), canine PLM (SEQ ID NO:3, GI 108084, Palmer et al, supra), human MAT-8 (SEQ ID NO:4, GI 1085026, Morrison et al, supra), rat CHIF (SEQ ID NO:5, GI 951423, Attali B et al (1995), supra), and mouse Na,K-ATPase .gamma.-subunit (SEQ ID NO:6, GI 51112, Mercer R W et al, supra). Sequences were aligned using the multisequence alignment program of DNASTar.TM. software (DNASTAR Inc, Madison, Wis.).

DEPR:

The present invention is based, in part, on the chemical and structural homology among, HPLP, PLM (SEQ ID NO:3, GI 108084; Palmer C et al, supra), MAT-8 (SEQ NO:4; GI 1085026; Morrison B et al (1995), supra), CHIF (SEQ ID NO:5; GI 951423, Attali B et al (1995), supra), and Na,K-ATPase .gamma.-subunit (SEQ ID NO:6, GI 51112, Mercer R W et al, supra). PLM, Mat-8, CHIF, and Na,K-ATPase .gamma.-subunit have, respectively, 46%, 31%, 25%, and 27% sequence identity to HPLP. The identity increases within the transmembrane domains of these proteins; the transmembrane domains of PLM, Mat-8, CHIF, and Na, K-ATPase .gamma.-subunit have, respectively, 60%, 45%, 40%, and 60% sequence identity with the predicted transmembrane domain of HPLP.

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Aug 31, 1999

DOCUMENT-IDENTIFIER: US 5945505 A

TITLE: Human phospholemann-like protein

BSPR:

The sodium potassium ATPase (Na,K-ATPase) .gamma.-subunit, formerly known as the Na,K-ATPase proteolipid, is a small membrane protein that co-purifies with the .alpha.- and .beta.-subunits of Na,K-ATPase (Mercer R W et al (1993) J Cell Biol 121:579-586). The .gamma.-subunit is a small membrane protein consisting of 58 amino acids with a single transmembrane domain. This transmembrane domain is structurally related to the transmembrane domains of other PLM-like proteins. The .gamma.-subunit may act as a regulator of the ATP-dependent ion channel activity of Na,K-ATPase.

BSPR:

The present invention features a novel human PLM-like protein, hereinafter referred to as HPLP, having chemical and structural homology to PLM, Mat-8, CHIF, and Na,K-ATPase .gamma.-subunit. Accordingly, the invention features a substantially purified HPLP, encoded by amino acid sequence of SEQ ID NO:1, having structural characteristics of the family of PLM-like proteins.

DRPR:

FIG. 2 shows the amino acid sequence alignments among HPLP (SEQ ID NO:1), canine PLM (SEQ ID NO:3, GI 108084, Palmer et al, supra), human MAT-8 (SEQ ID NO:4, GI 1085026, Morrison et al, supra), rat CHIF (SEQ ID NO:5, GI 951423, Attali B et al (1995), supra), and mouse Na,K-ATPase .gamma.-subunit (SEQ ID NO:6, GI 51112, Mercer R W et al, supra). Sequences were aligned using the multisequence alignment program of DNASTar.TM. software (DNASTAR Inc, Madison, Wis.).

DEPR:

The present invention is based, in part, on the chemical and structural homology among, HPLP, PLM (SEQ ID NO:3, GI 108084; Palmer C et al, supra), MAT-8 (SEQ NO:4; GI 1085026; Morrison B et al (1995), supra), CHIF (SEQ ID NO:5; GI 951423, Attali B et al (1995), supra), and Na,K-ATPase .gamma.-subunit (SEQ ID NO:6, GI 51112, Mercer R W et al, supra). PLM, Mat-8, CHIF, and Na,K-ATPase .gamma.-subunit have, respectively, 46%, 31%, 25%, and 27% sequence identity to HPLP. The identity increases within the transmembrane domains of these proteins; the transmembrane domains of PLM, Mat-8, CHIF, and Na, K-ATPase .gamma.-subunit have, respectively, 60%, 45%, 40%, and 60% sequence identity with the predicted transmembrane domain of HPLP.

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Synthesis of a functional F0 sector of the Escherichia coli H+- ATPase does not require synthesis of the alpha or beta subunits of F1.

Fillingame RH; Porter B; Hermolin J; White LK

Journal of bacteriology (UNITED STATES) Jan 1986, 165 (1) p244-51,

ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: GM-23105, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The uncB, E, F, and H genes of the Escherichia coli unc operon were cloned behind the lac promoter of plasmid pUC9, generating plasmid pBP101. These unc loci code, respectively, for the chi, omega, and psi subunits of the F0 sector and the delta subunit of the F1 sector of the H+-ATP synthase complex. Induction of expression of the four unc genes by the addition of isopropyl-beta-D-thiogalactoside resulted in inhibition of growth. During isopropyl-beta-D-thiogalactoside induction, the three subunits of F0 were integrated into the cytoplasmic membrane with a resultant increase in H⁺ permeability. A functional F0 was formed from plasmid pBP101 in a genetic background lacking all eight of the unc structural genes coding the F1F0 complex. In the unc deletion background, a reasonable correlation was observed between the amount of F0 incorporated into the membrane and the function measured, i.e., high-affinity binding of F1 and rate of F0-mediated H⁺ translocation. This correlation indicates that most or all of the F0 assembled in the membrane is active. Although the F0 assembled under these conditions binds F1, only partial restoration of NADH-dependent or ATP-dependent quenching of quinacrine fluorescence was observed with these membranes. Proteolysis of a fraction of the psi subunit may account for this partial deficiency. The experiments described demonstrate that a functional F0 can be assembled in vivo in E. coli strains lacking genes for the alpha, beta, gamma, and epsilon subunits of F1.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Escherichia coli--enzymology--EN; *H(+) -Transporting ATP Synthase--biosynthesis--BI; Bacterial Proteins--metabolism--ME; Chromosome Deletion ; Escherichia coli--genetics--GE; Genes, Bacterial; H(+) -Transporting ATP Synthase--genetics--GE; NAD--pharmacology--PD; Plasmids

CAS Registry No.: 0 (Bacterial Proteins); 0 (Plasmids); 53-84-9 (NAD)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19860220

E. coli leads to resistance to aminoglycoside antibiotics.

Humbert R; Altendorf K

Department of Biological Sciences, Stanford University, California
94305-5020.

Journal of bacteriology (UNITED STATES) Mar 1989, 171 (3) p1435-44,
ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: GM 18539, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

A strain of *Escherichia coli* which was derived from a gentamicin-resistant clinical isolate was found to be cross-resistant to neomycin and streptomycin. The molecular nature of the genetic defect was found to be an **insertion** of two GC base pairs in the uncG gene of the mutant. The **insertion** led to the production of a truncated **gamma** subunit of 247 amino acids in length instead of the 286 amino acids that are present in the normal **gamma** subunit. A **plasmid** which carried the ATP synthase genes from the mutant produced resistance to aminoglycoside antibiotics when it was introduced into a strain with a chromosomal **deletion** of the ATP synthase genes. Removal of the genes coding for the beta and epsilon subunits abolished antibiotic resistance coded by the mutant **plasmid**. The relationship between antibiotic resistance and the **gamma** subunit was investigated by testing the antibiotic resistance of **plasmids** carrying various combinations of unc genes. The presence of genes for the F0 portion of the ATP synthase in the presence or absence of genes for the **gamma** subunit was not sufficient to cause antibiotic resistance. alpha, beta, and truncated **gamma** subunits were detected on washed membranes of the mutant by immunoblotting. The first 247 amino acid residues of the **gamma** subunit may be sufficient to allow its association with other F1 subunits in such a way that the proton gate of F0 is held open by the mutant F1.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Antibiotics, Aminoglycoside--pharmacology--PD; **Escherichia coli*--enzymology--EN; *H(+)--Transporting ATP Synthase--genetics--GE; Amino Acid Sequence; Base Sequence; Drug Resistance, Microbial; *Escherichia coli*--drug effects--DE; *Escherichia coli*--genetics--GE; Genes, Bacterial; Genes, Structural; Genotype; Macromolecular Systems; Molecular Sequence Data; **Plasmids**; Species Specificity

CAS Registry No.: 0 (Antibiotics, Aminoglycoside); 0 (Macromolecular Systems); 0 (Plasmids)

Enzyme No.: EC 3.6.1.34 (H(+)--Transporting ATP Synthase)

Record Date Created: 19890414

Two genes, atpC1 and atpC2 , for the gamma subunit of Arabidopsis thaliana chloroplast ATP synthase .

Inohara N; Iwamoto A; Moriyama Y; Shimomura S; Maeda M; Futai M
Department of Organic Chemistry and Biochemistry, Osaka University,
Japan.

Journal of biological chemistry (UNITED STATES) Apr 25 1991, 266 (12)
p7333-8, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Arabidopsis thaliana has two genes (atpC1, atpC2) coding for **gamma** subunits of chloroplast ATP synthase. The atpC1 and atpC2 were cloned and sequenced. They had no introns within the reading frames and coded for proteins of 373 and 386 amino acid residues, respectively, including putative transit sequences (50 and 60 amino acid residues, respectively). In contrast, the spinach **gamma** subunit gene had two introns within the reading frame. The mature sequences coded by the two genes of *A. thaliana* (atpC1, 323 residues; atpC2, 326 residues) were homologous with that of spinach (J. Miki, M. Maeda, Y. Mukohata, and M. Futai (1988) FEBS Lett. 232, 221-226): the homologies of **gamma** subunits coded by atpC1 and atpC2 were 72%, those of the subunits coded by atpC1 and spinach cDNA were 84%, and those of the proteins coded by atpC2 and spinach cDNA were 71%. Like the spinach subunit, the **gamma** subunits coded by the two genes had unique regulatory domains not found in mitochondrial or bacterial subunits. Poly(A)+ mRNAs corresponding to atpC1 (1.5 kilobases) and atpC2 (2.5 kilobases) were detected in illuminated plants, the amount of the former being at least 140 times that of the latter. The atpC1 mRNA was not found in dark-adapted plants. Nuclear protein(s) specifically bound to the upstream region of atpC1 was detected by gel shift assay and its binding was shown to be inhibited by the GT-1 element of the gene encoding the ribulose-1,5-bisphosphate carboxylase small subunit, which is **expressed** under illumination (P. J. Green, S. A. Kay, and N. H. Chau (1987) EMBO J. 6, 2543-2549). Consistent with these findings, an increased amount of the **gamma** subunit was detected immunochemically in illuminated plants.

Tags: Support, Non-U.S. Gov't

Descriptors: *Chloroplasts--enzymology--EN; *H(+) -Transporting ATP Synthase--genetics--GE; *Plants--enzymology--EN; Amino Acid Sequence; DNA --genetics--GE; Immunochemistry; Molecular Sequence Data; Nuclear Proteins --analysis--AN; Plants--genetics--GE; RNA, Messenger--genetics--GE; Sequence Alignment; Sequence Homology, Nucleic Acid

Molecular Sequence Databank No.: GENBANK/J05760; GENBANK/J05761

CAS Registry No.: 0 (Nuclear Proteins); 0 (RNA, Messenger); 9007-49-2 (DNA)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Gene Symbol: ist/GeneSymbol atpC1; ist/GeneSymbol atpC2

Record Date Created: 19910529

948698 94043360 PMID: 8227057

**Gene structure of human mitochondrial ATP synthase gamma -subunit.
Tissue specificity produced by alternative RNA splicing.**

Matsuda C; Endo H; Ohta S; Kagawa Y

Department of Biochemistry, Jichi Medical School, Tochigi, Japan.

Journal of biological chemistry (UNITED STATES) Nov 25 1993, 268 (33)
p24950-8, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

We completely sequenced the human gene for ATP synthase **gamma** -subunit, which was approximately 23 kilobases long and was composed of 10 exons. Exons 1 and 2 encoded the N-terminal presequence required for mitochondrial import, while exons 9 and 10 encoded the C-terminal portions of mature protein. Enzymatic amplification of human heart and liver cDNAs using the polymerase chain reaction revealed two mRNA transcripts that were predicted to encode two 30-kDa isoforms of the **gamma** -subunit, which differed by the addition of a single amino acid (Asp273) at the C terminus of the liver type isoform. These two mRNA transcripts of the heart (H) type and liver (L) type were generated by alternative splicing of an exon. The same alternative splicing event was observed in bovine tissue. In human tissues, the H type mRNA devoid of exon 9 was **expressed** specifically in the heart and skeletal muscle, which require rapid energy supply. The L type mRNA was **expressed** in the brain, liver, kidney etc. Both transcripts were **expressed** in the skin, intestine, stomach, and aorta. This tissue specificity of transcript heterogeneity suggests the distinct functional or regulatory roles of the **gamma** -subunit isoforms in the catalysis of ATP synthase. This is the first report on tissue-specific isoforms generated by alternative splicing in an energy transducing mitochondrial protein.

Tags: Animal; Human; Support, Non-U.S. Gov't

Descriptors: *Alternative Splicing; *H(+) -Transporting ATP Synthase --genetics--GE; *Mitochondria--enzymology--EN; Amino Acid Sequence; Base Sequence; Blotting, Southern; Cattle; Molecular Sequence Data; Oligodeoxyribonucleotides; RNA, Messenger--genetics--GE; RNA, Messenger --metabolism--ME; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/D16561; GENBANK/D16562; GENBANK/D16563

CAS Registry No.: 0 (Oligodeoxyribonucleotides); 0 (RNA, Messenger)

Enzyme No.: EC 3.6.1.34 (H(+)-Transporting ATP Synthase)

Record Date Created: 19931220

Chromosomal localization of genes required for the terminal steps of oxidative metabolism: alpha and gamma subunits of ATP synthase and the phosphate carrier.

Jabs EW; Thomas PJ; Bernstein M; Coss C; Ferreira GC; Pedersen PL

Department of Pediatrics and Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287.

Human genetics (GERMANY) May 1994, 93 (5) p600-2, ISSN 0340-6717

Journal Code: GED

Contract/Grant No.: CA 10951, CA, NCI; HG 00373, HG, NHGRI

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The terminal steps of oxidative phosphorylation include transport of phosphate and ADP into the mitochondrial matrix, synthesis of ATP in the matrix, and transport of the product ATP into the cytosol where it can be utilized to perform **cellular** work. Three nuclear genome encoded membrane proteins, namely, the phosphate carrier (PHC), the adenine nucleotide carrier (ANT), and the ATP synthase complex, consisting of at least 13 individual subunits, catalyze these reactions. The locations of the alpha and **gamma** subunits of the mitochondrial ATP synthase complex and the mitochondrial phosphate carrier, PHC, on human chromosomes were determined using cloned rat liver cDNA as probes. Human homologues of the alpha subunit are on chromosomes 9 and 18, the **gamma** subunit are on chromosomes 10 and 14, and the PHC was localized to chromosome 12.

Tags: Animal; Human; Support, U.S. Gov't, P.H.S.

Descriptors: *Carrier Proteins--genetics--GE; *Chromosome Mapping; *H(+) -Transporting ATP Synthase--genetics--GE; *Membrane Proteins--genetics --GE; *Phosphates--metabolism--ME; Adenine Nucleotide Translocase--genetics --GE; Adenine Nucleotide Translocase--metabolism--ME; CHO Cells ; Carrier Proteins--metabolism--ME; DNA Probes; H(+) -Transporting ATP Synthase --metabolism--ME; Hamsters; Membrane Proteins--metabolism--ME; Oxidative Phosphorylation

CAS Registry No.: 0 (Carrier Proteins); 0 (DNA Probes); 0 (Membrane Proteins); 0 (Phosphates); 0 (phosphate-binding proteins)

Enzyme No.: EC 2.7.7.- (Adenine Nucleotide Translocase); EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19940602

Mitochondrial ATP synthase : dramatic Mg²⁺-induced alterations in the structure and function of the F1- ATPase moiety.

Pedersen PL; Williams N; Hullihen J

Laboratory for Molecular and Cellular Bioenergetics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Biochemistry (UNITED STATES) Dec 29 1987, 26 (26) p8631-7, ISSN 0006-2960 Journal Code: AOG

Contract/Grant No.: CA 10951, CA, NCI

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The ATPase activity of the F1 moiety of rat liver ATP synthase is **inactivated** when incubated prior to assay at 25 degrees C in the presence of MgCl₂. The concentration of MgCl₂ (130 microM) required to induce half-maximal **inactivation** is over 30 times higher than the apparent Km (MgCl₂) during catalysis. Moreover, the relative efficacy of divalent cations in inducing **inactivation** during prior incubation follows an order significantly different from that promoting catalysis. **Inactivation** of F1-ATPase activity by Mg²⁺ is accompanied by the dramatic dissociation from the F1 complex of alpha subunits and part of the **gamma**-subunit population. The latter form a precipitate while the beta, delta, and epsilon subunits, and the remaining part of the **gamma**-subunit population, remain soluble. Dissociation is not a sudden "all or none" event but parallels loss of ATPase activity until alpha subunits have almost completely dissociated together with about 50% of the **gamma**-subunit population. Mg²⁺-induced loss of F1-ATPase activity cannot be prevented by including either the hydrolytic substrates ATP, GTP, or ITP in the incubation medium or the product ADP. Ethylenediaminetetraacetic acid, mercaptoethanol, and dithiothreitol are also ineffective in preventing loss of ATPase activity. Significantly, KPi at high concentration (greater than or equal to 200 mM) is effective in partially protecting F1 against **inactivation**. However, the most effective means of preventing Mg²⁺-induced **inactivation** of F1-ATPase activity is to rebind F1 to its F0 moiety in F1-depleted particles. When bound to F0, F1 is protected completely against divalent cation induced **inactivation**. (ABSTRACT TRUNCATED AT 250 WORDS)

Tags: Animal; Support, U.S. Gov't, P.H.S.

Descriptors: *H(+) -Transporting ATP Synthase--metabolism--ME; *Magnesium --pharmacology--PD; *Mitochondria, Liver--enzymology--EN; Cations, Divalent ; Kinetics; Macromolecular Systems; Magnesium Chloride; Rats

CAS Registry No.: 0 (Cations, Divalent); 0 (Macromolecular Systems); 7439-95-4 (Magnesium); 7786-30-3 (Magnesium Chloride)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19880506

Isolation of a cDNA clone for the gamma subunit of the chloroplast ATP synthase of Chlamydomonas reinhardtii: import and cleavage of the precursor protein.

Yu LM; Merchant S; Theg SM; Selman BR

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison 53706.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 1988, 85 (5) p1369-73, ISSN 0027-8424

Journal Code: PV3

Contract/Grant No.: GM 31384, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

A cDNA library from Chlamydomonas reinhardtii, constructed in the phage **expression vector** lambda gt11, was probed with antiserum directed against the nuclear-encoded **gamma** subunit of the chloroplast H⁺-transporting ATP synthase [ATP phosphohydrolase (H⁺-transporting) or chloroplast coupling factors 0 and 1, EC 3.6.1.34] of C. reinhardtii. A cDNA was isolated and transcribed in vitro. The transcript was translated in vitro and immunoprecipitated with anti- **gamma** -subunit serum to yield a product that coelectrophoresed with the immunoprecipitated product from in vitro-translated polyadenylylated RNA. These proteins were larger than the mature **gamma** subunit, either immunoprecipitated as chloroplast coupling factor 1 or as the individual subunit. Thus, the **gamma** subunit is synthesized as a precursor of greater molecular weight in C. reinhardtii. Furthermore, the precursor protein encoded by the cDNA is imported into pea chloroplasts and processed to a lower molecular weight polypeptide that coelectrophoreses with mature C. reinhardtii **gamma** subunit. The largest cDNA isolated is about the same length as the corresponding mRNA (approximately equal to 1900 bases long) and probably contains the entire coding region. Southern blot analyses revealed restriction fragment length polymorphisms and that the **gamma** subunit is probably encoded by an intron-containing single-copy gene.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Chlamydomonas--genetics--GE; *Chloroplasts--enzymology--EN; *H(+) -Transporting ATP Synthase--genetics--GE; Biological Transport; Cloning, Molecular; DNA--genetics--GE; Genes, Structural; H(+) -Transporting ATP Synthase--metabolism--ME; Immunosorbent Techniques; Protein Processing, Post-Translational; RNA, Messenger--genetics--GE; Translation, Genetic

CAS Registry No.: 0 (RNA, Messenger); 9007-49-2 (DNA)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19880401

The membrane bound ATP synthase of Escherichia coli: a review of structural and functional analyses of the atp operon.

von Meyenburg K; Jorgensen BB; Nielsen J; Hansen FG; Michelsen O
Tokai journal of experimental and clinical medicine (JAPAN) 1982, 7
Suppl p23-31, ISSN 0385-0005 Journal Code: VZM

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The structure of the atp operon, which contains the genes for the eight subunits alpha, beta, **gamma**, delta, epsilon, a, b and c of the membrane bound ATP synthase of Escherichia coli as determined by genetic experiments and DNA sequencing, is reviewed. The localization of transcription signals, namely of one major and two minor promoters, as well as the determination of the stoichiometry of the subunits (alpha:beta: **gamma**:delta:epsilon:a:b:c = 3:3:1:1:1:1:2:12-15) is summarized.

Tags: Support, Non-U.S. Gov't

Descriptors: *Escherichia coli--enzymology--EN; *Genes, Bacterial;
*Genes, Structural; *Multienzyme Complexes--genetics--GE; *Operon;
*Phosphotransferases--genetics--GE; Base Sequence; Cell Membrane
--enzymology--EN; Cloning, Molecular; DNA Restriction Enzymes; Escherichia
coli--genetics--GE; Macromolecular Systems; Molecular Weight; Mutation;
Transcription, Genetic

CAS Registry No.: 0 (Macromolecular Systems); 0 (Multienzyme Complexes)

Enzyme No.: EC 2.7 (Phosphotransferases); EC 2.7.4.- (ATP synthetase complex); EC 3.1.21 (DNA Restriction Enzymes)

Record Date Created: 19831021

The atp operon: nucleotide sequence of the region encoding the alpha-subunit of Escherichia coli ATP - synthase .

Gay NJ; Walker JE

Nucleic acids research (ENGLAND) May 11 1981, 9 (9) p2187-94, ISSN 0305-1048 Journal Code: O8L

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Part of the atp (or unc) operon encoding the alpha, beta, gamma , delta, and epsilon subunits of Escherichia coli ATP-synthase has been cloned into the plasmid pACYC 184. The DNA coding for the largest of these proteins, the alphas subunit, has been sequenced by cloning into the bacteriophage M13 and sequencing with dideoxy nucleotide chain terminators. It comprises 1539 nucleotides corresponding to a protein of 513 amino acids.

Descriptors: *Escherichia coli--enzymology--EN; *Multienzyme Complexes --genetics--GE; *Operon; *Phosphotransferases--genetics--GE; Adenosine Diphosphate--genetics--GE; Base Sequence; Cloning, Molecular; DNA Restriction Enzymes; DNA, Bacterial; Deoxyribonuclease EcoRI; Plasmids

Molecular Sequence Databank No.: GENBANK/J01594; GENBANK/J01595; GENBANK/K02181; GENBANK/V00264; GENBANK/V00313; GENBANK/X00771

CAS Registry No.: 0 (DNA, Bacterial); 0 (Multienzyme Complexes); 0 (Plasmids); 58-64-0 (Adenosine Diphosphate)

Enzyme No.: EC 2.7 (Phosphotransferases); EC 2.7.4.- (ATP synthetase complex); EC 3.1.21 (DNA Restriction Enzymes); EC 3.1.21.- (Deoxyribonuclease EcoRI); EC 3.1.21.- (endodeoxyribonuclease HpaI)

Record Date Created: 19820109

The 20 C-terminal amino acid residues of the chloroplast ATP synthase gamma subunit are not essential for activity.

Sokolov M; Lu L; Tucker W; Gao F; Gegenheimer PA; Richter ML
Department of Molecular Biosciences, The University of Kansas, Lawrence,
Kansas 66045, USA.

Journal of biological chemistry (UNITED STATES) May 14 1999, 274 (20)
p13824-9, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

It has been suggested that the last seven to nine amino acid residues at the C terminus of the gamma subunit of the ATP synthase act as a spindle for rotation of the gamma subunit with respect to the alpha beta subunits during catalysis (Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) Nature 370, 621-628). To test this hypothesis we selectively **deleted** C-terminal residues from the chloroplast gamma subunit, two at a time starting at the sixth residue from the end and finishing at the 20th residue from the end. The mutant gamma genes were overexpressed in Escherichia coli and assembled with a native alpha₃beta₃ complex. All the mutant forms of gamma assembled as effectively as the wild-type gamma. **Deletion** of the terminal 6 residues of gamma resulted in a significant increase (>50%) in the Ca-dependent ATPase activity when compared with the wild-type assembly. The increased activity persisted even after **deletion** of the C-terminal 14 residues, well beyond the seven residues proposed to form the spindle. Further **deletions** resulted in a decreased activity to approximately 19% of that of the wild-type enzyme after **deleting** all 20 C-terminal residues. The results indicate that the tip of the gammaC terminus is not essential for catalysis and raise questions about the role of the C terminus as a spindle for rotation.

Tags: Animal; Support, U.S. Gov't, Non-P.H.S.

Descriptors: *Chloroplasts--enzymology--EN; *H(+) -Transporting ATP Synthase--chemistry--CH; Amino Acid Sequence; Cattle; Enzyme Inhibitors --metabolism--ME; Escherichia coli; H(+) -Transporting ATP Synthase --antagonists and inhibitors--AI; H(+) -Transporting ATP Synthase--genetics --GE; Mitochondria, Heart--enzymology--EN; Models, Molecular; Molecular Sequence Data; Mutagenesis, Site-Directed; Protein Conformation; Protein Folding; Spinach--enzymology--EN; Structure-Activity Relationship

CAS Registry No.: 0 (Enzyme Inhibitors)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19990617

Purification and reconstitution into proteoliposomes of the F1F0 ATP synthase from the obligately anaerobic gram-positive bacterium Clostridium thermoautotrophicum.

Das A; Ivey DM; Ljungdahl LG

Department of Biochemistry and Molecular Biology, University of Georgia, Athens 30602, USA.

Journal of bacteriology (UNITED STATES) Mar 1997, 179 (5) p1714-20,
ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: 5 R01 DK 27363 16, DK, NIDDK; AM27323, AM, NIADDK

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The proton-translocating F1F0 ATP synthase from Clostridium thermoautotrophicum was solubilized from cholate-washed membranes with Zwittergent 3-14 at 58 degrees C and purified in the presence of octylglucoside by sucrose gradient centrifugation and ion-exchange chromatography on a DEAE-5PW column. The purified enzyme hydrolyzed ATP at a rate of 12.6 micromol min(-1) mg(-1) at 58 degrees C and pH 8.5. It was composed of six different polypeptides with molecular masses of 60, 50, 32, 19, 17, and 8 kDa. These were identified as alpha, beta, gamma, delta, epsilon, and c subunits, respectively, as their N-terminal amino acid sequences matched the deduced N-terminal amino acid sequences of the corresponding genes of the atp operon sequenced from Clostridium thermoaceticum (GenBank accession no. U64318), demonstrating the close similarity of the F1F0 complexes from C. thermoaceticum and C. thermoautotrophicum. Four of these subunits, alpha, beta, gamma, and epsilon, constituted the F1-ATPase purified from the latter bacterium. The delta subunit could not be found in the purified F1 although it was present in the F1F0 complex, indicating that the F0 moiety consisted of the delta and the c subunits and lacked the a and b subunits found in many aerobic bacteria. The c subunit was characterized as N,N'-dicyclohexylcarbodiimide reactive. The F1F0 complex of C. thermoautotrophicum consisting of subunits alpha, beta, gamma, delta, epsilon, and c was reconstituted with phospholipids into proteoliposomes which had ATP-Pi exchange, carbonylcyanide p-trifluoromethoxy-phenylhydrazone-stimulated ATPase, and ATP-dependent proton-pumping activities. Immunoblot analyses of the subunits of ATP synthases from C. thermoautotrophicum, C. thermoaceticum, and Escherichia coli revealed antigenic similarities among the F1 subunits from both clostridia and the beta subunit of F1 from E. coli.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Clostridium--enzymology--EN; *H(+) -Transporting ATP Synthase--isolation and purification--IP; *H(+) -Transporting ATP Synthase --metabolism--ME; *Liposomes--chemistry--CH; *Proteolipids; Adenosine Triphosphate--metabolism--ME; Amino Acid Sequence ; Carbonyl Cyanide p-Trifluoromethoxyphenylhydrazone--pharmacology--PD; Dicyclohexylcarbodiimide--metabolism--ME; Dicyclohexylcarbodiimide--pharmacology--PD; Escherichia coli--enzymology--EN; H(+) -Transporting ATP Synthase--chemistry--CH; H(+) -Transporting ATP Synthase--immunology--IM; Immunoblotting; Molecular Sequence Data; Molecular Weight; Proton Pump

Molecular Sequence Databank No.: GENBANK/U64318

CAS Registry No.: 0 (Liposomes); 0 (Proteolipids); 0 (Proton Pump); 0 (proteoliposomes); 370-86-5 (Carbonyl Cyanide p-Trifluoromethoxyphenyl hydrazone); 538-75-0 (Dicyclohexylcarbodiimide); 56-65-5 (Adenosine Triphosphate)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19970325

Acidic stimulation induces a negative regulatory factor that affects alternative exon selection in vitro in human ATP synthase gamma-subunit pre-mRNA.

Hayakawa M; Endo H; Hamamoto T; Kagawa Y

Department of Biochemistry, Jichi Medical School, Minamikawachi-machi, Tochigi, Kawachi-gun, 329-0498, Japan.

Biochemical and biophysical research communications (UNITED STATES) Oct 20 1998, 251 (2) p603-8, ISSN 0006-291X Journal Code: 9Y8

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Tissue-specific alternative RNA splicing in human Flgamma pre-mRNA produces muscle- and nonmuscle-type isoforms. Muscle-specific exclusion of exon 9 of the Flgamma gene is cell -specifically induced by acidic treatment of human fibrosarcoma HT1080 and rhabdomyosarcoma KYM-1 cells . We constructed an Flgamma minigene containing parts of exon 8, intron 8, and exon 9 of the human Flgamma gene and then analyzed a negative factor that inhibited inclusion of exon 9 via an in vitro splicing assay using acid-stimulated HT1080 cell nuclear extract. In vitro splicing of the Flgamma minigene, similarly to the beta-globin minigene used as a control, was observed in HeLa cell nuclear extract. Next, we performed supplemental experiments using HeLa and HT1080 cell nuclear extracts. The splicing reaction of the Flgamma minigene was specifically inhibited by supplementation with nuclear extract from acid-stimulated HT1080 cells , whereas that of human beta-globin was not inhibited. These results indicated that acidic stimulation induced a negative factor that blocked inclusion of alternatively spliced exon in the Flgamma minigene in vitro, and a regulatory factor acted in a sequence-specific manner for muscle-specific alternative splicing in Flgamma pre-mRNA. Copyright 1998 Academic Press.

Tags: Human; Support, Non-U.S. Gov't

Descriptors: Alternative Splicing; *Exons; *Gene Expression Regulation, Enzymologic; *H(+) -Transporting ATP Synthase--genetics--GE; *Hydrogen-Ion Concentration; *RNA Precursors--genetics--GE; *Tissue Extracts --pharmacology--PD; Base Sequence; Cell Nucleus--metabolism--ME; Fibrosarcoma; Gene Expression Regulation, Neoplastic; Genes, Synthetic; H(+) -Transporting ATP Synthase--biosynthesis--BI; Hela Cells; Introns; Macromolecular Systems; Molecular Sequence Data; RNA Precursors--metabolism --ME; Rhabdomyosarcoma; Tumor Cells, Cultured

CAS Registry No.: 0 (Macromolecular Systems); 0 (RNA Precursors); 0 (Tissue Extracts)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Sequence analysis of the atp operon of Clostridium acetobutylicum DSM 792 encoding the FOF1 ATP synthase .

Externbrink T; Hujer S; Winzer K; Durre P

Mikrobiologie und Biotechnologie, Universitat Ulm, Germany.

DNA sequence (SWITZERLAND) 2000, 11 (1-2) p109-18, ISSN 1042-5179,

Journal Code: A9H

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The atp gene region of Clostridium acetobutylicum DSM 792 has been fully sequenced. It contains the FOF1 ATPase genes in the order atpIBEFHAGDC, whose products share high sequence homology to the respective proteins of a variety of other bacteria. It is the first such sequence available for a mesophilic Clostridium. Significant differences to other reported atp operons are a distal transcription start point 219 bp upstream of the translation start point and a second transcription initiation site (without corresponding promoter sequence) upstream of atpE, indicating posttranscriptional processing for massive **expression** of this gene product.

Tags: Support, Non-U.S. Gov't

Descriptors: *Clostridium--enzymology--EN; *Genes, Bacterial;
*H(+) -Transporting ATP Synthase--genetics--GE; *Operon; Amino Acid Sequence
; Base Sequence; Clostridium--genetics--GE; DNA, Bacterial; Molecular
Sequence Data; Sequence Analysis, DNA; Transcription, Genetic; Translation,
Genetic

Molecular Sequence Databank No.: GENBANK/AF101055

CAS Registry No.: 0 (DNA, Bacterial)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 20001124

On the role of Arg-210 and Glu-219 of subunit a in proton translocation
by the *Escherichia coli* FOF1- ATP synthase .

Valiyaveetil FI; Fillingame RH

Department of Biomolecular Chemistry, University of Wisconsin Medical School, Madison, Wisconsin 53706, USA.

Journal of biological chemistry (UNITED STATES) Dec 19 1997, 272 (51)

p32635-41, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM23105, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

A strain of *Escherichia coli* was constructed which had a complete deletion of the chromosomal uncB gene encoding subunit a of the FOF1-ATP synthase. Gene replacement was facilitated by a selection protocol that utilized the sacB gene of *Bacillus subtilis* cloned in a kanamycin resistance cartridge (Ried, J. L., and Collmer, A. (1987) Gene (Amst.) 57, 239-246). F0 subunits b and c inserted normally into the membrane in the DeltauncB strain. This observation confirms a previous report (Hermolin, J., and Fillingame, R. H. (1995) J. Biol. Chem. 270, 2815-2817) that subunit a is not required for the insertion of subunits b and c. The DeltauncB strain has been used to characterize mutations in Arg-210 and Glu-219 of subunit a, residues previously postulated to be essential in proton translocation. The aE219G and aE219K mutants grew on a succinate carbon source via oxidative phosphorylation and membranes from these mutants exhibited ATPase-coupled proton translocation (i.e. ATP driven 9-amino-6-chloromethoxyacridine quenching responses that were 60-80% of wild type membranes). We conclude that the aGlu-219 residue cannot play a critical role in proton translocation. The aR210A mutant did not grow on succinate and membranes exhibited no ATPase-coupled proton translocation. However, on removal of F1 from membrane, the aR210A mutant F0 was active in passive proton translocation, i.e. in dissipating the DeltapH normally established by NADH oxidation with these membrane vesicles. aR210A membranes with F1 bound were also proton permeable. Arg-210 of subunit a may play a critical role in active H⁺ transport that is coupled to ATP synthesis or hydrolysis, but is not essential for the translocation of protons across the membranes.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Arginine--metabolism--ME; **Escherichia coli*--enzymology--EN ; *Glutamic Acid--metabolism--ME; *H(+)--Transporting ATP Synthase --metabolism--ME; Chromosomes, Bacterial; H(+)--Transporting ATP Synthase --chemistry--CH; Ion Transport; Mutation; Protons

CAS Registry No.: 0 (Protons); 56-86-0 (Glutamic Acid); 7004-12-8 (Arginine)

Enzyme No.: EC 3.6.1.34 (H(+)--Transporting ATP Synthase)

Record Date Created: 19980122

Nuclear respiratory factor 1 activation sites in genes encoding the gamma -subunit of ATP synthase , eukaryotic initiation factor 2 alpha, and tyrosine aminotransferase. Specific interaction of purified NRF-1 with multiple target genes.

Chau CM; Evans MJ; Scarpulla RC

Department of Cell, Molecular, and Structural Biology, Northwestern University Medical School, Chicago, Illinois 60611.

Journal of biological chemistry (UNITED STATES) Apr 5 1992, 267 (10)
p6999-7006, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM32525-09, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Transcription factor nuclear respiratory factor 1 (NRF-1) was originally identified as an activator of the cytochrome c gene and subsequently found to stimulate transcription through specific sites in other nuclear genes whose products function in the mitochondria. These include subunits of the cytochrome oxidase and reductase complexes and a component of the mitochondrial DNA replication machinery. Here we establish that a functional recognition site for NRF-1 is present in the ATP synthase **gamma**-subunit gene extending the proposed respiratory role of NRF-1 to complex V. In addition, biologically active NRF-1 sites are found in genes encoding the eukaryotic translation initiation factor 2 alpha-subunit and tyrosine aminotransferase, both of which participate in the rate-limiting step of their respective pathways of protein biosynthesis and tyrosine catabolism. The recognition sites from each of these genes form identical complexes with NRF-1 as established by competition binding assays, methylation interference footprinting, and UV-induced DNA cross-linking. Cloned oligomers of each NRF-1 binding site also stimulate the activity of a truncated cytochrome c promoter in transfected **cells**. The NRF-1 binding activities for the various target sites copurified approximately 33,000-fold and resided in a single protein of 68 kDa. These observations further support a role for NRF-1 in the **expression** of nuclear respiratory genes and suggest it may help coordinate respiratory metabolism with other biosynthetic and degradative pathways.

Tags: Animal; Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: *H(+) -Transporting ATP Synthase--genetics--GE;

*Transcription Factors--metabolism--ME; *Tyrosine Transaminase--genetics--GE; *eIF-2--genetics--GE; Base Sequence; Binding Sites; Cattle; DNA, Mitochondrial--genetics--GE; DNA, Mitochondrial--radiation effects--RE; DNA-Binding Proteins--metabolism--ME; Electrophoresis, Polyacrylamide Gel; Hela **Cells**; Methylation; Mice; Molecular Sequence Data; Promoter Regions (Genetics); Rats; Transfection; Ultraviolet Rays

CAS Registry No.: 0 (DNA, Mitochondrial); 0 (DNA-Binding Proteins); 0 (Transcription Factors); 0 (eIF-2)

Enzyme No.: EC 2.6.1.5 (Tyrosine Transaminase); EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19920428

679127 98151240 PMID: 9492276

Topology of subunit a of the Escherichia coli ATP synthase .

Jager H; Birkenhager R; Stalz WD; Altendorf K; Deckers-Hebestreit G
Universitat Osnabruck, Fachbereich Biologie/Chemie, Abteilung
Mikrobiologie, Germany.

European journal of biochemistry (GERMANY) Jan 15 1998; 251 (1-2)
p122-32, ISSN 0014-2956 Journal Code: EMZ

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The antigenic determinants of mAbs against subunit a of the Escherichia coli ATP synthase were mapped by ELISA using overlapping synthetic decapeptides. For two of the mAbs the epitopes are E4NMTPQD10 (GDH 14-5C6) and V29DPQ32 (GDH 8-8B3). Binding of these mAbs to membrane vesicles of different orientation revealed that both epitopes are accessible in vesicles with inside-out orientation. These results demonstrate that at least the N-terminal amino acids 1-32 of subunit a are located at the cytoplasmic side of the membrane. A further determination of the topology of subunit a was performed by inserting the reporter epitope DYKDDDDK (FLAG epitope) at different positions of the polypeptide chain. 10 of 13 insertions led to a functional F0F1 ATP synthase and allowed specific detection of the modified subunit a by immunoblotting using an mAb against the FLAG epitope. In addition, polyclonal anti-FLAG IgG was applied for the recognition of the mutant FLAG epitope DYKDDVDK. Cells carrying this mutant FLAG epitope at the C terminus of subunit a were able to grow on succinate as sole carbon and energy source, revealing a functional ATP synthase, in contrast to those carrying the original FLAG epitope at the same position. Binding studies with membrane vesicles of different orientation and anti-FLAG Ig demonstrated that both termini of the protein are located at the cytoplasmic side of the membrane, indicating that an even number of membrane-spanning segments is present in subunit a. In addition, insertion of two FLAG epitopes in tandem after K66, or one epitope after H95, and Q181 revealed that the polypeptide regions including these residues are accessible from the cytoplasmic surface of the membrane. These results support the view that the polypeptide chain of subunit a traverses the membrane six times.

Tags: Support, Non-U.S. Gov't

Descriptors: *Escherichia coli--enzymology--EN; *H(+) -Transporting ATP Synthase--immunology--IM; *H(+) -Transporting ATP Synthase--metabolism--ME ; Amino Acid Sequence; Antibodies, Monoclonal; Antibody Specificity; Cell Membrane--metabolism--ME; Cytoplasm--metabolism--ME; Epitopes; H(+) -Transporting ATP Synthase--genetics--GE; Histidine; Molecular Sequence Data; Recombinant Proteins--genetics--GE; **Recombinant Proteins**--immunology--IM ; **Recombinant Proteins**--metabolism--ME

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Epitopes); 0 (Recombinant Proteins); 7006-35-1 (Histidine)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19980323

Characterization of a b₂delta complex from Escherichia coli ATP synthase .

Dunn SD; Chandler J

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Journal of biological chemistry (UNITED STATES) Apr 10 1998, 273 (15) p8646-51, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The delta subunit of Escherichia coli ATP synthase has been **expressed** and purified, both as the intact polypeptide and as delta', a proteolytic fragment composed of residues 1-134. The solution structure of delta' as a five-helix bundle has been previously reported (Wilkens, S., Dunn, S. D., Chandler, J., Dahlquist, F. W., and Capaldi, R. A. (1997) Nat. Struct. Biol. 4, 198-201). The delta subunit, in conjunction with delta-depleted F1-ATPase, was fully capable of reconstituting energy-dependent fluorescence quenching in membrane vesicles that had been depleted of F1. A complex of delta with the cytoplasmic domain of the b subunit of F0 was demonstrated and characterized by analytical ultracentrifugation using bST34-156, a form of the b domain lacking aromatic residues. Molecular weight determination by sedimentation equilibrium supported a b₂delta subunit stoichiometry. The sedimentation coefficient of the complex, 2.1 S, indicated a frictional ratio of approximately 2, suggesting that delta and the b dimer are arranged in an end-to-end rather than side-by-side manner. These results indicate the feasibility of the b₂delta complex reaching from the membrane to the membrane-distal portion of the F1 sector, as required if it is to serve as a second stalk.

Tags: Support, Non-U.S. Gov't

Descriptors: *Escherichia coli--enzymology--EN; *H(+)-Transporting ATP Synthase--chemistry--CH; Cloning, Molecular; DNA Primers; H(+)-Transporting ATP Synthase--isolation and purification--IP; Kinetics; Macromolecular Systems; Molecular Weight; Quinacrine; **Recombinant Proteins**--chemistry--CH; **Recombinant Proteins**--isolation and purification--IP; Spectrometry, Fluorescence

CAS Registry No.: 0 (DNA Primers); 0 (Macromolecular Systems); 0 (Recombinant Proteins); 83-89-6 (Quinacrine)

Enzyme No.: EC 3.6.1.34 (H(+)-Transporting ATP Synthase)

Record Date Created: 19980514

Lengthening the second stalk of F(1)F(0) ATP synthase in Escherichia coli.

Sorgen PL; Bubb MR; Cain BD

Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32610, USA.

Journal of biological chemistry (UNITED STATES) Dec 17 1999, 274 (51)

p36261-6, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM43495, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

In *Escherichia coli* F(1)F(0) ATP synthase, the two b subunits dimerize forming the peripheral second stalk linking the membrane F(0) sector to F(1). Previously, we have demonstrated that the enzyme could accommodate relatively large **deletions** in the b subunits while retaining function (Sorgen, P. L., Caviston, T. L., Perry, R. C., and Cain, B. D. (1998) J. Biol. Chem. 273, 27873-27878). The manipulations of b subunit length have been extended by construction of **insertion** mutations into the uncF(b) gene adding amino acids to the second stalk. Mutants with **insertions** of seven amino acids were essentially identical to wild type strains, and mutants with **insertions** of up to 14 amino acids retained biologically significant levels of activity. Membranes prepared from these strains had readily detectable levels of F(1)F(0)-ATPase activity and proton pumping activity. However, the larger **insertions** resulted in decreasing levels of activity, and immunoblot analysis indicated that these reductions in activity correlated with reduced levels of b subunit in the membranes. Addition of 18 amino acids was sufficient to result in the loss of F(1)F(0) ATP synthase function. Assuming the predicted alpha-helical structure for this area of the b subunit, the 14-amino acid **insertion** would result in the addition of enough material to lengthen the b subunit by as much as 20 A. The results of both **insertion** and **deletion** experiments support a model in which the second stalk is a flexible feature of the enzyme rather than a rigid rod-like structure.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Bacterial Proteins--metabolism--ME; **Escherichia coli*--enzymology--EN; *H(+)-Transporting ATP Synthase--metabolism--ME; Amino Acid Sequence; Bacterial Proteins--chemistry--CH; Bacterial Proteins--genetics--GE; Base Sequence; H(+)-Transporting ATP Synthase--chemistry--CH; H(+)-Transporting ATP Synthase--genetics--GE; Molecular Sequence Data; Mutagenesis, Insertional; Mutation

CAS Registry No.: 0 (Bacterial Proteins)

Enzyme No.: EC 3.6.1.34 (H(+)-Transporting ATP Synthase)

Record Date Created: 20000127

Molecular and functional characterization of the *Salmonella typhimurium* invasion genes *invB* and *invC*: homology of InvC to the F0F1 ATPase family of proteins.

Eichelberg K; Ginocchio CC; Galan JE
Department of Microbiology, School of Medicine, SUNY Stony Brook
11794-5222.

Journal of bacteriology (UNITED STATES) Aug 1994, 176 (15) p4501-10,
ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: AI30492, AI, NIAID

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Entry into intestinal epithelial cells is an essential step in the pathogenesis of *Salmonella* infections. Our laboratory has previously identified a **genetic** locus, *inv*, that is necessary for efficient entry of *Salmonella typhimurium* into cultured epithelial cells. We have carried out a molecular and functional analysis of *invB* and *invC*, two members of this locus. The **nucleotide sequence** of these genes indicated that *invB* and *invC* encode polypeptides with molecular masses of 15 and 47 kDa, respectively. Polypeptides with the predicted sizes were observed when these genes were expressed under the control of a T7 promoter. Strains carrying nonpolar mutations in these genes were constructed, and their phenotypes were examined in a variety of assays. A mutation in *invC* rendered *S. typhimurium* defective in their ability to enter cultured epithelial cells, while mutations in *invB* did not. Comparison of the predicted sequences of *InvB* and *InvC* with translated sequences in **GenBank** revealed that these polypeptides are similar to the *Shigella* spp. proteins Spal5 and Spa47, which are involved in the surface presentation of the invasion protein antigens (Ipa) of these organisms. In addition, *InvC* showed significant similarity to a protein family which shares **sequence homology** with the catalytic beta subunit of the F0F1 ATPase from a number of microorganisms. Consistent with this finding, purified preparations of *InvC* showed significant ATPase activity. Site-directed mutagenesis of a residue essential for the catalytical function of this family of proteins resulted in a protein devoid of ATPase activity and unable to complement an *invC* mutant of *S. typhimurium*. These results suggest that *InvC* may energize the protein export apparatus encoded in the *inv* locus which is required for the surface presentation of determinants needed for the entry of *Salmonella* species into mammalian cells. The role of *InvB* in this process remains uncertain.

Tags: Comparative Study; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: Genes, Bacterial-- **genetics** --GE; *H(+-)Transporting ATP Synthase-- **genetics** --GE; **Salmonella typhimurium*-- **genetics** --GE; Adenosine Triphosphate--metabolism--ME; Amino Acid **Sequence**; Bacterial Proteins-- **genetics** --GE; Base **Sequence**; Cells, Cultured; DNA Mutational Analysis; **Genetic** Complementation Test; Molecular **Sequence** Data; Polarity of Translation; Protein Binding; *Salmonella typhimurium*--pathogenicity--PY; **Sequence** Analysis, DNA; **Sequence** Homology, Amino Acid; Virulence-- **genetics** --GE

Molecular Sequence Databank No.: GENBANK/U08279

CAS Registry No.: 0 (Bacterial Proteins); 0 (*invB* protein); 56-65-5 (Adenosine Triphosphate)

Enzyme No.: EC 3.6.1.- (*invC* protein); EC 3.6.1.34 (H(+-)Transporting ATP Synthase)

Gene Symbol: ist/GeneSymbol *invB*; ist/GeneSymbol *invC*

Record Date Created: 19940830

**Subunit 9 of the mitochondrial ATP synthase of *Trypanosoma brucei* is
nuclearly encoded and developmentally regulated.**

Chi TB; Brown B SV; Williams N

Department of Microbiology, State University of New York at Buffalo,
14214, USA.

Molecular and biochemical parasitology (NETHERLANDS) Apr 1 1998, 92

(1) p29-38, ISSN 0166-6851 Journal Code: NOR

Contract/Grant No.: R01AI33694, AI, NIAID

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

We have previously shown that the mitochondrial ATP synthase is developmentally regulated through the life cycle of *Trypanosoma brucei*. The mechanism of this regulation is as yet unknown. We are currently examining regulation of **expression** of several key subunits of the ATP synthase to investigate this mechanism. In the work presented here, we have cloned, sequenced, and confirmed the identity of the ATPase subunit 9 homologue from *T. brucei*. The ATPase subunit 9 gene that we have identified from *T. brucei* has between 40 and 600% identity with subunit 9 from a variety of organisms. This gene possesses a putative mitochondrial import sequence at the N terminus of the encoded protein sequence. The protein **expressed** from this gene by in vitro transcription/translation comigrates with native protein isolated from inner mitochondrial membrane vesicles from *T. brucei*. We have shown that the cDNA identifies a copy of this gene in the nuclear genome, but does not identify a similar gene in kinetoplast DNA (kDNA) prepared from *T. brucei*. This gene does not show homology to any published sequence data from maxicircle DNA or edited maxicircle derived sequences. Steady state transcripts of a single size have been identified by Northern analysis and demonstrate significant developmental regulation through the *T. brucei* life cycle. Northern analysis and quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) results show that the transcript is 10-14-fold higher in procyclic form than in early and late bloodstream forms.

Tags: Animal; Comparative Study; Support, U.S. Gov't, P.H.S.

Descriptors: **Cell** Nucleus--genetics--GE; *Gene **Expression** Regulation; *H(+) -Transporting ATP Synthase--genetics--GE; *Mitochondria--enzymology --EN; **Trypanosoma brucei* brucei--genetics--GE; Amino Acid Sequence; Blotting, Southern; Carbodiimides--pharmacology--PD; Cell Compartmentation --genetics--GE; Cell Cycle; Cell Differentiation; Cloning, Molecular; Enzyme Inhibitors--pharmacology--PD; Gene Dosage; Genes, Protozoan; H(+) -Transporting ATP Synthase--antagonists and inhibitors--AI; H(+) -Transporting ATP Synthase--isolation and purification--IP; Intracellular Membranes--enzymology--EN; Molecular Sequence Data; Polymerase Chain Reaction; **Recombinant** Proteins --isolation and purification--IP; **Recombinant** Proteins--metabolism--ME; Sequence Analysis, DNA; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/AF014058

CAS Registry No.: 0 (Carbodiimides); 0 (Enzyme Inhibitors); 0 (Recombinant Proteins); 86332-16-3 (N-cyclohexyl-N'-(4-dimethylamino-alpha-naphthyl)carbodiimide)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19980622

Subunit epsilon of the Escherichia coli ATP synthase : novel insights into structure and function by analysis of thirteen mutant forms.

Xiong H; Zhang D; Vik SB

Department of Biological Sciences, Southern Methodist University, Dallas, Texas 75275, USA.

Biochemistry (UNITED STATES) Nov 17 1998, 37 (46) p16423-9, ISSN 0006-2960 Journal Code: A0G

Contract/Grant No.: GM40508, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Structural models of subunit epsilon of the ATP synthase from Escherichia coli have been determined recently by NMR [Wilkens et al. (1995) Nat. Struct. Biol. 2, 961-967] and by X-ray crystallography [Uhlén et al. (1997) Structure 5, 1219-1230], revealing a two-domain protein. In this study, six new epsilon mutants were constructed and analyzed: Y63A, D81A, T82A, and three truncated mutants, tr80(S), tr94(LAS), and tr117(AS). Seven mutants constructed previously were also analyzed: E31A, E59A, S65A, E70A, T77A, R58A, and D81A/R85A. Subunits were purified by isoelectric focusing from extracts of **cells** that overproduced these 13 mutants. F1 was prepared lacking subunit epsilon by immobilized-Ni affinity chromatography. Three mutants, E70A, S65A, and E31A, showed somewhat higher affinities and extents of inhibition than the wild type. Three mutants, T82A, R85A, and tr94(LAS), showed both lower affinities and extents of inhibition, over the concentration range tested. Two showed no inhibition, D81A and tr80(S). The others, T77A, Y63A, E59A, and tr117(AS), showed lower affinities than wild type, but the extents of inhibition were nearly normal. Results indicate that the C-terminal domain of subunit epsilon contributes to inhibition of ATP hydrolysis, but it is not necessary for ATP-driven proton translocation. Interactions with subunit gamma are likely to involve a surface containing residues S65, E70, T77, D81, and T82, while residues R85 and Y63 are likely to be important in the conformation of subunit epsilon.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: Escherichia coli--enzymology--EN; *H(+) -Transporting ATP Synthase--chemistry--CH; *H(+) -Transporting ATP Synthase--genetics--GE; *Mutagenesis, Insertional ; Adenosine Triphosphate --antagonists and inhibitors--AI; Alanine--genetics--GE; Amino Acid Substitution--genetics --GE; Cell Membrane--enzymology--EN; Cell Membrane--genetics--GE; Escherichia coli--growth and development--GD; H(+) -Transporting ATP Synthase--isolation and purification--IP; Hydrolysis--drug effects--DE; Models, Molecular; Peptide Fragments--chemistry--CH; Peptide Fragments --genetics--GE; Proton Pump--drug effects--DE; Proton Pump--genetics--GE; Structure-Activity Relationship

CAS Registry No.: 0 (Peptide Fragments); 0 (Proton Pump); 56-65-5 (Adenosine Triphosphate); 6898-94-8 (Alanine)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19981217

Insertion scanning mutagenesis of subunit a of the F1F0 ATP synthase near His245 and implications on gating of the proton channel.

Vik SB; Patterson AR; Antonio BJ

Department of Biological Sciences, Southern Methodist University Dallas,
Texas 75275, USA. svik@mail.smu.edu

Journal of biological chemistry (UNITED STATES) Jun 26 1998, 273 (26)
p16229-34, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM40508, GM, NIGMS

Erratum in J Biol Chem 1998 Aug 21;273(34) 22159

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Subunit a of the *E. coli* F1F0 ATP synthase was probed by **insertion** scanning mutagenesis in a region between residues Glu219 and His245. A series of single amino acid **insertions**, of both alanine and aspartic acid, were constructed after the following residues: 225, 229, 233, 238, 243, and 245. The mutants were tested for growth yield, binding of F1 to membranes, dicyclohexylcarbodiimide sensitivity of ATPase activity, ATP-driven proton translocation, and passive proton permeability of membranes stripped of F1. Significant loss of function was seen only with **insertions** after positions 238 and 243. In contrast, both **insertions** after residue 225 and the alanine **insertion** after residue 245 were nearly identical in function to the wild type. The other **insertions** showed an intermediate loss of function. Missense mutations of His245 to serine and cysteine were nonfunctional, while the W241C mutant showed nearly normal ATPase function. Replacement of Leu162 by histidine failed to suppress the 245 mutants, but chemical rescue of H245S was partially successful using acetate. An interaction between Trp241 and His245 may be involved in gating a "half-channel" from the periplasmic surface of F0 to Asp61 of subunit a.

Tags: Comparative Study; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: H(+) -Transporting ATP Synthase--genetics--GE; *Histidine --genetics--GE; *Ion Channel Gating--genetics--GE; *Mutagenesis, **Insertional**; *Proton Pump--genetics--GE; Amino Acid Sequence; Amino Acid Substitution; Base Sequence; *Escherichia coli*; H(+) -Transporting ATP Synthase--metabolism--ME; *Haemophilus influenzae*; Molecular Sequence Data; Protein Conformation; Restriction Mapping; Structure-Activity Relationship; *Vibrio*

CAS Registry No.: 0 (Proton Pump); 7006-35-1 (Histidine)

Enzyme No.: EC 3.6.1.34 (H(+)-Transporting ATP Synthase)

Record Date Created: 19980803

09869316 98053844 PMID: 9393695

In vitro Tn7 mutagenesis of *Haemophilus influenzae* Rd and characterization of the role of atpA in transformation.

Gwinn ML; Stellwagen AE; Craig NL; Tomb JF; Smith HO

Department of Molecular Biology and Genetics, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205, USA. mlgwinn@tigr.org

Journal of bacteriology (UNITED STATES) Dec 1997, 179 (23) p7315-20, ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: GM48251, GM, NIGMS; GM53824, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

***Haemophilus influenzae* Rd** is a gram-negative bacterium capable of natural DNA transformation. The competent state occurs naturally in late exponential growth or can be induced by a nutritional downshift or by transient anaerobiosis. The genes cya, crp, topA, and sxy (tfoX) are known to function in the regulation of competence development. The phosphoenolpyruvate:carbohydrate phosphotransferase system functions to maintain levels of cyclic AMP necessary for competence development but is not directly involved in regulation. The exact signal(s) for competence and the genes that mediate the signal(s) are still unknown. In an effort to find additional regulatory genes, *H. influenzae* Rd was mutated by using an in vitro Tn7 system and screened for mutants with a reduced ability to induce the competence-regulatory gene, comA. Insertions in atpA, a gene coding for the alpha subunit of the F1 cytoplasmic domain of the ATP synthase, reduce transformation frequencies about 20-fold and cause a significant reduction in expression of competence-regulatory genes, while the expression of constitutive competence genes is only minimally affected. In addition, we found that an insertion in atpB, which encodes the a subunit of the F0 membrane-spanning domain, has a similar effect on transformation frequencies.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: H(+) -Transporting ATP Synthase--genetics--GE; * ***Haemophilus influenzae*--genetics--GE**; *Transformation, Genetic; Bacterial Proteins --genetics--GE; DNA Transposable Elements; DNA-Binding Proteins--genetics --GE; Genes, Bacterial; ***Haemophilus influenzae*--enzymology--EN**; Ketone Oxidoreductases--genetics--GE; Mutagenesis, Insertional; Phenotype; Phosphoenolpyruvate Sugar Phosphotransferase System--genetics--GE; Phosphotransferases (Nitrogenous Group Acceptor)--genetics--GE; Selection (Genetics)

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA Transposable Elements); 0 (DNA-Binding Proteins); 0 (comA protein)

Enzyme No.: EC 1.2. (Ketone Oxidoreductases); EC 1.2.7.1 (pyruvate synthase); EC 2.7.1.- (Phosphoenolpyruvate Sugar Phosphotransferase System); EC 2.7.3 (Phosphotransferases (Nitrogenous Group Acceptor)); EC 2.7.3.9 (phosphoenolpyruvate-protein phosphotransferase); EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19971230

4/9/9

DIALOG(R) File 155: MEDLINE(R)

09809432 98298134 PMID: 9632681

Insertion scanning mutagenesis of subunit a of the F1F0 ATP synthase near His245 and implications on gating of the proton channel.

Vik SB; Patterson AR; Antonio BJ

Department of Biological Sciences, Southern Methodist University Dallas, Texas 75275, USA. svik@mail.smu.edu

Journal of biological chemistry (UNITED STATES) Jun 26 1998, 273 (26) p16229-34, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM40508, GM, NIGMS

Erratum in J Biol Chem 1998 Aug 21;273(34) 22159

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Intergenic suppression of the gammaM23K uncoupling mutation in F0F1 ATP synthase by betaGlu-381 substitutions: the role of the beta380DELSEED386 segment in energy coupling.

Ketchum CJ; Al-Shawi MK; Nakamoto RK

Department of Molecular Physiology and Biological Physics, University of Virginia, P.O. Box 10011, Charlottesville, VA 22906-0011, USA.

Biochemical journal (ENGLAND) Mar 1 1998, 330 (Pt 2) p707-12, ISSN 0264-6021 Journal Code: 9YO

Contract/Grant No.: GM50957, GM, NIGMS; GM52502, GM, NIGMS; HL07284, HL, NHLBI

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

We previously demonstrated that the Escherichia coli F0F1-ATP synthase mutation, gammaM23K, caused increased energy of interaction between gamma- and beta-subunits which was correlated to inefficient coupling between catalysis and transport [Al-Shawi, Ketchum and Nakamoto (1997) J. Biol. Chem. 272, 2300-2306]. Based on these results and the X-ray crystallographic structure of bovine F1-ATPase [Abrahams, Leslie, Lutter and Walker (1994) Nature (London) 370, 621-628] gammaM23K is believed to form an ionized hydrogen bond with betaGlu-381 in the conserved beta380DELSEED386 segment. In this report, we further test the role of gamma-beta-subunit interactions by introducing a series of substitutions for betaGlu-381 and gammaArg-242, the residue which forms a hydrogen bond with betaGlu-381 in the wild-type enzyme. betaE381A, D, and Q were able to restore efficient coupling when co- expressed with gammaM23K. All three mutations reversed the increased transition state thermodynamic parameters for steady state ATP hydrolysis caused by gammaM23K. betaE381K by itself caused inefficient coupling, but opposite from the effect of gammaM23K, the transition state thermodynamic parameters were lower than wild-type. These results suggest that the betaE381K mutation perturbs the gamma-beta-subunit interaction and the local conformation of the beta380DELSEED386 segment in a specific way that disrupts the communication of coupling information between transport and catalysis. betaE381A, L, K, and R, and gammaR242L and E mutations perturbed enzyme assembly and stability to varying degrees. These results provide functional evidence that the beta380DELSEED386 segment and its interactions with the gamma-subunit are involved in the mechanism of coupling.

Tags: Animal; Support, U.S. Gov't, P.H.S.

Descriptors: *H(+) -Transporting ATP Synthase--genetics--GE; *Suppression, Genetic; Amino Acid Substitution; Arginine--genetics--GE; Arginine--metabolism--ME; Cattle; Crystallography, X-Ray; Energy Metabolism; Escherichia coli--enzymology--EN; Escherichia coli--genetics--GE; Glutamic Acid--genetics--GE; Glutamic Acid--metabolism--ME; H(+) -Transporting ATP Synthase--metabolism--ME; Models, Molecular; Mutagenesis, Site-Directed; Protein Conformation; Structure-Activity Relationship; Thermodynamics

CAS Registry No.: 56-86-0 (Glutamic Acid); 7004-12-8 (Arginine)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19980416

Deletions in the second stalk of F1F0- ATP synthase in Escherichia coli.

Sorgen PL; Caviston TL; Perry RC; Cain BD

Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32610, USA.

Journal of biological chemistry (UNITED STATES) Oct 23 1998, 273 (43)

p27873-8, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM43495, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

In Escherichia coli F1F0-ATP synthase, the two b subunits form the second stalk spanning the distance between the membrane F0 sector and the bulk of F1. Current models predict that the stator should be relatively rigid and engaged in contact with F1 at fixed points. To test this hypothesis, we constructed a series of **deletion** mutations in the uncF(b) gene to remove segments from the middle of the second stalk of the subunit. Mutants with **deletions** of 7 amino acids were essentially normal, and those with **deletions** of up to 11 amino acids retained considerable activity. Membranes prepared from these strains had readily detectable levels of F1-ATPase activity and proton pumping activity. Removal of 12 or more amino acids resulted in loss of oxidative phosphorylation. Levels of membrane-associated F1-ATPase dropped precipitously for the longer **deletions**, and immunoblot analysis indicated that reductions in activity correlated with reduced levels of b subunit in the membranes. Assuming the likely alpha-helical conformation for this area of the b subunit, the 11-amino acid **deletion** would result in shortening the subunit by approximately 16 A. Since these **deletions** did not prevent the b subunit from participating in productive interactions with F1, we suggest that the b subunit is not a rigid rodlike structure, but has an inherent flexibility compatible with a dynamic role in coupling.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Bacterial Proteins--metabolism--ME; *Escherichia coli --enzymology--EN; *H(+) -Transporting ATP Synthase--metabolism--ME; Adenosine Triphosphate--metabolism--ME; Bacterial Proteins--genetics--GE; Base Sequence; Escherichia coli--genetics--GE; H(+) -Transporting ATP Synthase--genetics--GE; Membranes--enzymology--EN; Models, Theoretical; Molecular Sequence Data; Mutagenesis; Permeability; Proton Pump; Sequence Deletion

CAS Registry No.: 0 (Bacterial Proteins); 0 (Proton Pump); 0 (uncF protein, Escherichia coli); 56-65-5 (Adenosine Triphosphate)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19981112

Rotation of the epsilon subunit during catalysis by Escherichia coli FOF1- ATP synthase .

Bulygin VV; Duncan TM; Cross RL

Department of Biochemistry and Molecular Biology, State University of New York Health Science Center, Syracuse, New York 13210, USA.

Journal of biological chemistry (UNITED STATES) Nov 27 1998, 273 (48)

p31765-9, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: GM 23152, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

We report evidence for catalysis-dependent rotation of the single epsilon subunit relative to the three catalytic beta subunits of functionally coupled, membrane-bound FOF1-ATP synthase. Cysteines substituted at beta380 and epsilon108 allowed rapid formation of a specific beta-epsilon disulfide cross-link upon oxidation. Consistent with a need for epsilon to rotate during catalysis, tethering epsilon to one of the beta subunits resulted in the inhibition of both ATP synthesis and hydrolysis. These activities were fully restored upon reduction of the beta-epsilon cross-link. As a more critical test for rotation, a subunit dissociation/reassociation procedure was used to prepare a beta-epsilon cross-linked hybrid F1 having epitope-tagged betaD380C subunits (betaflag) exclusively in the two noncross-linked positions. This allowed the beta subunit originally aligned with epsilon to form the cross-link to be distinguished from the other two betas. The cross-linked hybrid was reconstituted with FO in F1-depleted membranes. After reduction of the beta-epsilon cross-link and a brief period of catalytic turnover, reoxidation resulted in a significant amount of betaflag in the beta-epsilon cross-linked product. In contrast, exposure to ligands that bind to the catalytic site but do not allow catalysis resulted in the subsequent cross-linking of epsilon to the original untagged beta. Furthermore, catalysis-dependent rotation of epsilon was prevented by prior treatment of membranes with N,N'-dicyclohexylcarbodiimide to block proton translocation through FO. From these results, we conclude that epsilon is part of the rotor that couples proton transport to ATP synthesis.

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Escherichia coli--enzymology--EN; *H(+) -Transporting ATP Synthase--chemistry--CH; *H(+) -Transporting ATP Synthase--metabolism--ME; Catalysis; Cross-Linking Reagents; Cysteine; Dithionitrobenzoic Acid --pharmacology--PD; Kinetics; Macromolecular Systems; Mutagenesis, Site-Directed; Protein Hybridization; **Recombinant Proteins**--chemistry--CH ; **Recombinant Proteins**--metabolism--ME; Rotation

CAS Registry No.: 0 (Cross-Linking Reagents); 0 (Macromolecular Systems); 0 (Recombinant Proteins); 52-90-4 (Cysteine); 69-78-3 (Dithionitrobenzoic Acid)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19981223

**Identification of subunit g of yeast mitochondrial F1F0- ATP synthase ,
a protein required for maximal activity of cytochrome c oxidase.**
Boyle GM; Roucou X; Nagley P; Devenish RJ; Prescott M
Department of Biochemistry, Monash University, Victoria, Australia.
European journal of biochemistry (GERMANY) Jun 1999, 262 (2) p315-23
, ISSN 0014-2956 Journal Code: EMZ
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Subfile: INDEX MEDICUS

By means of a yeast genome database search, we have identified an open reading frame located on chromosome XVI of *Saccharomyces cerevisiae* that encodes a protein with 53% amino acid similarity to the 11.3-kDa subunit g of bovine mitochondrial F1F0-ATP synthase. We have designated this ORF ATP20, and its product subunit g. A null mutant strain, constructed by **insertion** of the HIS3 gene into the coding region of ATP20, retained oxidative phosphorylation function. Assembly of F1F0-ATP synthase in the atp20-null strain was not affected in the absence of subunit g and levels of oligomycin-sensitive ATP hydrolase activity in mitochondria were normal. Immunoprecipitation of F1F0-ATP synthase from mitochondrial lysates prepared from atp20-null **cells expressing** a variant of subunit g with a hexahistidine motif indicated that this polypeptide was associated with other well-characterized subunits of the yeast complex. Whilst mitochondria isolated from the atp20-null strain had the same oxidative phosphorylation efficiency (ATP : O₂) as that of the control strain, the atp20-null strain displayed approximately a 30% reduction in both respiratory capacity and ATP synthetic rate. The absence of subunit g also reduced the activity of cytochrome c oxidase, and altered the kinetic control of this complex as demonstrated by experiments titrating ATP synthetic activity with cyanide. These results indicate that subunit g is associated with F1F0-ATP synthase and is required for maximal levels of respiration, ATP synthesis and cytochrome c oxidase activity in yeast.

Tags: Animal; Support, Non-U.S. Gov't

Descriptors: *Cytochrome-c Oxidase--metabolism--ME; *H(+) -Transporting ATP Synthase--metabolism--ME; *Saccharomyces cerevisiae--enzymology--EN; Amino Acid Sequence; Base Sequence; Cattle; Cytochrome-c Oxidase--chemistry --CH; DNA Primers; H(+) -Transporting ATP Synthase--chemistry--CH; Membrane Proteins--chemistry--CH; Membrane Proteins--metabolism--ME; Molecular Sequence Data; Oxidative Phosphorylation; Sequence Homology, Amino Acid

CAS Registry No.: 0 (DNA Primers); 0 (Membrane Proteins)

Enzyme No.: EC 1.9.3.1 (Cytochrome-c Oxidase); EC 3.6.1.34
(H(+) -Transporting ATP Synthase)

Record Date Created: 19990624

Escherichia coli ATP synthase alpha subunit Arg-376: the catalytic site arginine does not participate in the hydrolysis/ synthesis reaction but is required for promotion to the steady state.

Le NP; Omote H; Wada Y; Al-Shawi MK; Nakamoto RK; Futai M

Division of Biological Sciences, The Institute of Scientific and Industrial Research, Osaka University, CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation, Ibaraki, Osaka 567-0047, Japan.

Biochemistry (UNITED STATES) Mar 14 2000, 39 (10) p2778-83, ISSN 0006-2960 Journal Code: A0G

Contract/Grant No.: GM-50957, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The three catalytic sites of the F(O)F(1) ATP synthase interact through a cooperative mechanism that is required for the promotion of catalysis. Replacement of the conserved alpha subunit Arg-376 in the Escherichia coli F(1) catalytic site with Ala or Lys resulted in turnover rates of ATP hydrolysis that were 2 x 10(3)-fold lower than that of the wild type. Mutant enzymes catalyzed hydrolysis at a single site with kinetics similar to that of the wild type; however, addition of excess ATP did not chase bound ATP, ADP, or Pi from the catalytic site, indicating that binding of ATP to the second and third sites failed to promote release of products from the first site. Direct monitoring of nucleotide binding in the alphaR376A and alphaR376K mutant F(1) by a tryptophan in place of betaTyr-331 (Weber et al. (1993) J. Biol. Chem. 268, 20126-20133) showed that the catalytic sites of the mutant enzymes, like the wild type, have different affinities and therefore, are structurally asymmetric. These results indicate that alphaArg-376, which is close to the beta- or gamma-phosphate group of bound ADP or ATP, respectively, does not make a significant contribution to the catalytic reaction, but coordination of the arginine to nucleotide filling the low-affinity sites is essential for promotion of rotational catalysis to steady-state turnover.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Arginine--metabolism--ME; *Catalytic Domain; *Escherichia coli--enzymology--EN; *H(+) -Transporting ATP Synthase--metabolism--ME; Amino Acid Substitution--genetics--GE; Arginine--genetics--GE; Binding Sites--genetics--GE; Catalysis; Catalytic Domain--genetics--GE; Escherichia coli--genetics--GE; H(+) -Transporting ATP Synthase--biosynthesis--BI; H(+) -Transporting ATP Synthase--genetics--GE; Hydrolysis; Kinetics; Mutagenesis, Insertional ; Mutagenesis, Site-Directed; Phosphates --metabolism--ME

CAS Registry No.: 0 (Phosphates); 7004-12-8 (Arginine)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 20000419

The gammaepsilon-c subunit interface in the ATP synthase of Escherichia coli. cross-linking of the epsilon subunit to the c subunit ring does not impair enzyme function, that of gamma to c subunits leads to uncoupling.

Schulenberg B; Aggeler R; Murray J; Capaldi RA
Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229, USA.

Journal of biological chemistry (UNITED STATES) Nov 26 1999, 274 (48)
p34233-7, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: HL 24526, HL, NHLBI

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Mutants with a cysteine residue in the gamma subunit at position 207 and the epsilon subunit at position 31 were expressed in combination with a c-dimer construct, which contains a single cysteine at position 42 of the second c subunit. These mutants are called gammaY207C/cc'Q42C and epsilonE31C/cc'Q42C, respectively. Cross-linking of epsilon to the c subunit ring was obtained almost to completion without significant effect on any enzyme function, i.e. ATP hydrolysis, ATP synthesis, and ATP hydrolysis-driven proton translocation were all close to that of wild type. The gamma subunit could also be linked to the c subunit ring in more than 90% yield, but this affected coupling. Thus, ATP hydrolysis was increased 2.5-fold, ATP synthesis was dramatically decreased, and ATP hydrolysis-driven proton translocation was abolished, as measured by the 9-amino-6-chloro-2-methoxyacridine quenching method. These results for epsilonE31C/cc'Q42C indicate that the c subunit ring rotates with the central stalk element. That the gamma-epsilon cross-linked enzyme retains ATPase activity also argues for a gammaepsilon-c subunit rotor. However, the uncoupling induced by cross-linking of gamma to the c subunit ring points to important conformational changes taking place in the gammaepsilon-c subunit interface during this. Blocking these structural changes by cross-linking leads to a proton leak within the F(0).

Tags: Support, U.S. Gov't, P.H.S.

Descriptors: *Escherichia coli--enzymology--EN; *H(+) -Transporting ATP Synthase--metabolism--ME; Amino Acid Substitution; Binding Sites--genetics--GE; Copper--chemistry--CH; Copper--pharmacology--PD; Cross-Linking Reagents--chemistry--CH; Enzyme Activation--drug effects--DE; H(+) -Transporting ATP Synthase--chemistry--CH; H(+) -Transporting ATP Synthase--genetics--GE; Mutagenesis, Site-Directed; Mutation; Peptide Fragments--chemistry--CH; Peptide Fragments--genetics--GE; Peptide Fragments--metabolism--ME

CAS Registry No.: 0 (Cross-Linking Reagents); 0 (Peptide Fragments); 7440-50-8 (Copper); 7758-89-6 (cuprous chloride)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19991229

Refolding of recombinant alpha and beta subunits of the Rhodospirillum rubrum F(0)F(1) ATP synthase into functional monomers that reconstitute an active alpha(1)beta(1)-dimer.

Du Z; Gromet-Elhanan Z

Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel.

European journal of biochemistry (GERMANY) Jul 1999, 263 (2) p430-7, ISSN 0014-2956 Journal Code: EMZ

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The alpha subunit from the Rhodospirillum rubrum F(0)F(1) ATP synthase (RrF(1)alpha) was over- ~~expressed~~ in unc operon- ~~deleted~~ Escherichia coli strains under various growth conditions only in insoluble inclusion bodies. The functional refolding of urea-solubilized RrF(1)alpha was followed by measuring its ability to stimulate the restoration of ATP synthesis and hydrolysis in beta-less R. rubrum chromatophores reconstituted with pure native or **recombinant** RrF(1)beta [Nathanson, L. & Gromet-Elhanan, Z. (1998) J. Biol. Chem. 273, 10933-10938]. The refolding efficiency was found to increase with decreasing RrF(1)alpha concentrations and required high concentrations of MgATP, saturating approximately 60% when 50 microgram protein.mL(-1) were refolded in presence of 50 mM MgATP. Size-exclusion HPLC of such refolded RrF(1)alpha revealed a 50-60% decrease in its aggregated form and a parallel appearance of its monomeric peak. RrF(1)beta refolded under identical conditions appeared almost exclusively as a monomer. This procedure enabled the isolation of large amounts of a stable RrF(1)alpha monomer, which stimulated the restoration of ATP synthesis and hydrolysis much more efficiently than the refolded alpha mixture, and bound ATP and ADP in a Mg-dependent manner. Incubation of both RrF(1)alpha and beta monomers, which by themselves had no ATPase activity, resulted in a parallel appearance of activity and assembled alpha(1)beta(1)-dimers, but showed no formation of alpha(3)beta(3)-hexamers. The RrF(1)-alpha(1)beta(1)-ATPase activity was, however, very similar to the activity observed in isolated native chloroplast CF(1)-alpha(3)beta(3), indicating that these dimers contain only the catalytic nucleotide-binding site at their alpha/beta interface. Their inability to associate into an alpha(3)beta(3)-hexamer seems therefore to reflect a much lower stability of the noncatalytic RrF(1) alpha/beta interface.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, Non-P.H.S.

Descriptors: H(+)-Transporting ATP Synthase--chemistry--CH; * **Recombinant Proteins**--chemistry--CH; *Rhodospirillum rubrum--enzymology--EN; Adenosine Triphosphate--pharmacology--PD; Dose-Response Relationship, Drug; Kinetics; Models, Chemical; Protein Folding; Time Factors

CAS Registry No.: 0 (Recombinant Proteins); 56-65-5 (Adenosine Triphosphate)

Enzyme No.: EC 3.6.1.34 (H(+)-Transporting ATP Synthase)

Record Date Created: 19990826

Purification and reconstitution into proteoliposomes of the F1F0 ATP synthase from the obligately anaerobic gram-positive bacterium Clostridium thermoautotrophicum.

Das A; Ivey DM; Ljungdahl LG

Department of Biochemistry and Molecular Biology, University of Georgia, Athens 30602, USA.

Journal of bacteriology (UNITED STATES) Mar 1997, 179 (5) p1714-20,
ISSN 0021-9193 Journal Code: HH3

Contract/Grant No.: 5 R01 DK 27363 16, DK, NIDDK; AM27323, AM, NIADDK

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The proton-translocating F1F0 ATP synthase from Clostridium thermoautotrophicum was solubilized from cholate-washed membranes with Zwittergent 3-14 at 58 degrees C and purified in the presence of octylglucoside by sucrose gradient centrifugation and ion-exchange chromatography on a DEAE-5PW column. The purified enzyme hydrolyzed ATP at a rate of 12.6 micromol min(-1) mg(-1) at 58 degrees C and pH 8.5. It was composed of six different polypeptides with molecular masses of 60, 50, 32, 19, 17, and 8 kDa. These were identified as alpha, beta, gamma, delta, epsilon, and c subunits, respectively, as their N-terminal amino acid sequences matched the deduced N-terminal amino acid sequences of the corresponding genes of the atp operon sequenced from Clostridium thermoaceticum (GenBank accession no. U64318), demonstrating the close similarity of the F1F0 complexes from C. thermoaceticum and C. thermoautotrophicum. Four of these subunits, alpha, beta, gamma, and epsilon, constituted the F1-ATPase purified from the latter bacterium. The delta subunit could not be found in the purified F1 although it was present in the F1F0 complex, indicating that the F0 moiety consisted of the delta and the c subunits and lacked the a and b subunits found in many aerobic bacteria. The c subunit was characterized as N,N'-dicyclohexylcarbodiimide reactive. The F1F0 complex of C. thermoautotrophicum consisting of subunits alpha, beta, gamma, delta, epsilon, and c was reconstituted with phospholipids into proteoliposomes which had ATP-Pi exchange, carbonylcyanide p-trifluoromethoxy-phenylhydrazone-stimulated ATPase, and ATP-dependent proton-pumping activities. Immunoblot analyses of the subunits of ATP synthases from C. thermoautotrophicum, C. thermoaceticum, and Escherichia coli revealed antigenic similarities among the F1 subunits from both clostridia and the beta subunit of F1 from E. coli.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Clostridium--enzymology--EN; *H(+) -Transporting ATP Synthase--isolation and purification--IP; *H(+) -Transporting ATP Synthase --metabolism--ME; *Liposomes--chemistry--CH; *Proteolipids; Adenosine Triphosphate--metabolism--ME; Amino Acid **Sequence**; Carbonyl Cyanide p-Trifluoromethoxyphenylhydrazone--pharmacology--PD; Dicyclohexylcarbodiimide--metabolism--ME; Dicyclohexylcarbodiimide--pharmacology--PD; Escherichia coli--enzymology--EN; H(+) -Transporting ATP Synthase--chemistry--CH; H(+) -Transporting ATP Synthase--immunology--IM; Immunoblotting; Molecular **Sequence** Data; Molecular Weight; Proton Pump

Molecular Sequence Databank No.: GENBANK/U64318

CAS Registry No.: 0 (Liposomes); 0 (Proteolipids); 0 (Proton Pump); 0 (proteoliposomes); 370-86-5 (Carbonyl Cyanide p-Trifluoromethoxyphenyl hydrazone); 538-75-0 (Dicyclohexylcarbodiimide); 56-65-5 (Adenosine Triphosphate)

Enzyme No.: EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19970325

Cloning of the cDNA for the human ATP synthase OSCP subunit (ATP50) by exon trapping and mapping to chromosome 21q22.1-q22.2.

Chen H; Morris MA; Rossier C; Blouin JL; Antonarakis SE

Department of Genetics and Microbiology, Geneva University Medical School, Switzerland.

Genomics (UNITED STATES) Aug 10 1995, 28 (3) p470-6, ISSN 0888-7543
Journal Code: GEN

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Exon trapping was used to clone portions of potential genes from human chromosome 21. One trapped **sequence** showed striking homology with the bovine and rat ATP synthase OSCP (oligomycin sensitivity conferring protein) subunit. We subsequently cloned the full-length human ATP synthase OSCP cDNA (GDB/HGMW approved name ATP50) from infant brain and muscle libraries and determined its **nucleotide** and deduced amino acid **sequence** (EMBL/ **GenBank** Accession No. X83218). The encoded polypeptide contains 213 amino acids, with more than 80% identity to bovine and murine ATPase OSCP subunits and over 35% identity to *Saccharomyces cerevisiae* and sweet potato sequences. The human ATP50 **gene** is located at 21q22.1-q22.2, just proximal to D21S17, in YACs 860G11 and 838C7 of the Chumakov et al. (Nature 359:380, 1992) YAC contig. The **gene** is expressed in all human tissues examined, most strongly in muscle and heart. This ATP50 subunit is a key structural component of the stalk of the mitochondrial respiratory chain F1F0-ATP synthase and as such may contribute in a **gene** dosage-dependent manner to the phenotype of Down syndrome (trisomy 21).

Tags: Animal; Human; Support, Non-U.S. Gov't

Descriptors: Adenosinetriphosphatase-- **genetics** --GE; *Chromosomes, Human, Pair 21; *H(+) -Transporting ATP Synthase-- **genetics** --GE; *Membrane Proteins-- **genetics** --GE; Amino Acid **Sequence**; Base **Sequence**; Blotting, Northern; Blotting, Southern; Cattle; Chromosome Mapping; Cloning, Molecular; DNA, Complementary; Exons; Infant; Molecular **Sequence** Data; Rats; **Sequence** Homology, Amino Acid

Molecular Sequence Databank No.: GENBANK/M18753; GENBANK/X83218; GENBANK/X83219; SWISSPROT/P09457; SWISSPROT/P13621; SWISSPROT/P22778; SWISSPROT/Q06647

CAS Registry No.: 0 (DNA, Complementary); 0 (Membrane Proteins); 0 (oligomycin sensitivity-conferring protein)

Enzyme No.: EC 3.6.1.3 (Adenosinetriphosphatase); EC 3.6.1.34 (H(+) -Transporting ATP Synthase)

Record Date Created: 19960103

WEST



Generate Collection

L4: Entry 10 of 51

File: USPT

Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214591 B1

TITLE: Methods for producing L-valine and L-leucine

BSPR:

H_{sup.+} -ATPase is a membrane-binding enzyme with approximately 500,000 KD in molecular weight, in which 8 types of subunits complicatedly associate, and functions to pump H_{sup.+} outside of cytoplasm through changes in the free energy caused by ATP hydrolyzation and to synthesize ATP utilizing a H_{sup.+} -concentration gradient between the inside and outside of cytoplasmic membrane caused by intracellular respiration. Additionally, this enzyme is divided into an F0 fraction, which is localized on the inner membrane and exhibits H_{sup.+} -transport activity, and an F1 fraction, which is localized in the membrane surface and catalyzes the decomposition and synthesis of ATP, and the F0 is composed of 3 types of subunits a, b and c, while the F1 is composed of 5 types of subunits .alpha., .beta., .gamma., .delta., .epsilon.. A strain which has a mutation in any of these subunits can be used as a H_{sup.+} -ATPase-deficient strain. The mutation of the H_{sup.+} -ATPase deficiency may include the expression of a mutant subunit, and the non-expression of subunits comprising H_{sup.+} -ATPase by the mutation at a promoter site.

DEPR:

E. coli AJ12631 was obtained by transducing atpA401, a mutant gene, encoding mutant alpha subunit of F1 of H_{sup.+} -ATPase derived from E. coli AN718 (CGSC6308) into E. coli W1485lip2 (ATCC25645) (see Japanese Patent Application Laid-Open No. 5-137568(1993)). In selection of a transduced strain with a H_{sup.+} -ATPase-deficient mutation, bgl gene positioned in the vicinity of atpA401 gene was used as a marker. Since the bgl gene encodes phospho-beta-glucosidase, E. coli having the wild-type bgl gene (bgl.sup.-) cannot assimilate salicin, whereas E. coli having the mutant bgl gene (bgl.sup.+) can grow utilizing salicin as the sole carbon source, so that the colonies of a salicin-assimilating strain make a bromothymol blue-added medium plate turn yellow by an organic acid produced by the strain. Therefore, if the mutant bgl gene (bgl.sup.30) and atpA401 gene are linked-transduced, a H_{sup.+} -ATPase-deficient mutant can be selected efficiently. First, the salicin-assimilating (bgl.sup.30) strain was isolated from E. coli AN718, and then AN718 (bgl.sup.+) was infected by Plkc, and E. coli W1485lip2 was transduced using the obtained lysate. For the resulting transductant, a lipoic acid requirement and H_{sup.+} -ATPase activity was determined to confirm the presence of lipoic acid-requiring and H_{sup.+} -ATPase-deficient mutations.

*Updated
6/5/03
RJZ*

SYSTEM:OS - DIALOG OneSearch

File 155: MEDLINE(R) 1966-2003/Jun W2

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*File 155: Medline has been reloaded and accession numbers have changed. Please see HELP NEWS 155.

File 5:Biosis Previews(R) 1969-2003/Jun W2

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File 34:SciSearch(R) Cited Ref Sci 1990-2003/Jun W1

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File 144:Pascal 1973-2003/May W4

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File 149:TGG Health&Wellness DB(SM) 1976-2003/Jun W1

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File 156:ToxFile 1965-2003/Jun W2

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*File 156: ToxFile has been reloaded. Accession numbers have changed. Please see HELP NEWS 156 for details.

File 159:Cancerlit 1975-2002/Oct

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*File 159: Cancerlit ceases updating with immediate effect.

Please see HELP NEWS.

File 162:Global Health 1983-2003/Apr

(c) 2003 CAB International

*File 162: Effective May 1, name changes from CAB Health to Global Health.

File 164:Allied & Complementary Medicine 1984-2003/Jun

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File 172:EMBASE Alert 2003/Jun W2

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File 266:FEDRIP 2003/Apr

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File 370:Science 1996-1999/Jul W3

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File 399:CA SEARCH(R) 1967-2003/UD=13824

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File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

(c) 1998 Inst for Sci Info

File 442:AMA Journals 1982-2003/Nov B1

(c) 2003 Amer Med Assn -FARS/DARS apply

File 444:New England Journal of Med. 1985-2003/Jun W2

(c) 2003 Mass. Med. Soc.

File 467:ExtraMED(tm) 2000/Dec
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*File 467: For information about updating status please see Help News467.

Set Items Description

?

?e pasteurellaceae

Ref	Items	RT	Index-term
E1	5		PASTEURELLACEAE
E2	4		PASTEURELLACEA
E3	21681	69	*PASTEURELLACEAE
E4	1		PASTEURELLACEAE (BACTERIA)
E5	554		PASTEURELLACEAE (PASTEURELLACEAE)
E6	12840		PASTEURELLACEAE (1992-)
E7	2		PASTEURELLACEAE --ANALYSIS --AN
E8	4		PASTEURELLACEAE --CHEMISTRY --CH
E9	31		PASTEURELLACEAE --CLASSIFICATION --CL
E10	1		PASTEURELLACEAE --CYTOLOGY --CY
E11	5		PASTEURELLACEAE --DRUG EFFECTS --DE
E12	24		PASTEURELLACEAE --GENETICS --GE

Enter P or PAGE for more

?e e3

Ref	Items	Type	RT	Index-term
R1	13641		69	*PASTEURELLACEAE
R2	0	S		From 1992 to mid 1998, the Biosystematic Code
R3	0	S		BC06703 was used to refer to this taxonomic
R4	0	S		category.
R5	0	U		BC=06703
R6	67483	B	8	FACULTATIVELY ANAEROBIC GRAM-NEGATIVE RODS
R7	76	E		DC=B3.40.40.70
R8	32	B	131	GRAM NEGATIVE FACULTATIVELY ANAEROBIC RODS
R9	722	N	8	ACTINOBACILLUS ACTINOMYCETEMCOMITANS
R10	386	N	2	ACTINOBACILLUS PLEUROPNEUMONIAE
R11	130	N	3	HAEMOPHILUS APHROPHILUS
R12	620	N	6	HAEMOPHILUS DUCREYI

Enter P or PAGE for more

?p

Ref	Items	Type	RT	Index-term
R13	337	N	3	HAEMOPHILUS INFLUENZAE TYPE A
R14	2331	N	6	HAEMOPHILUS INFLUENZAE TYPE B
R15	410	N	3	HAEMOPHILUS PARAINFLUENZAE
R16	43	N	3	HAEMOPHILUS PARAPHROPHILUS
R17	47	N	3	HAEMOPHILUS PARASUIS
R18	11	N	3	HAEMOPHILUS SEGNIS
R19	100	N	3	HAEMOPHILUS SOMNUS
R20	638	N	3	PASTEURELLA HAEMOLYTICA
R21	1272	N	5	PASTEURELLA MULTOCIDA
R22	4	N	2	PASTEURELLA PISCICIDA
R23	1523	N	4	ACTINOBACILLUS
R24	18757	N	14	HAEMOPHILUS

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?p

Ref	Items	Type	RT	Index-term
R25	10592	N	10	HAEMOPHILUS INFLUENZAE
R26	2656	N	5	PASTEURELLA
R27	70	X		DC=B3.440.450.600.
R28	70	X		DC=B3.660.250.550.
R29	291	B	340	GAMMA PROTEOBACTERIA
R30	198	B	258	GRAM-NEGATIVE FACULTATIVELY ANAEROBIC RODS
R31	3656	N	15	ACTINOBACILLUS
R32	1480	N	9	ACTINOBACILLUS ACTINOMYCETEMCOMITANS

R33 524 N 12 ACTINOBACILLUS PLEUROPNEUMONIAE
R34 19897 N 21 HAEMOPHILUS
R35 518 N 12 HAEMOPHILUS DUCREYI
R36 9684 N 12 HAEMOPHILUS INFLUENZAE

Enter P or PAGE for more

?p

Ref	Items	Type	RT	Index-term
R37	358	N	15	HAEMOPHILUS INFLUENZAE TYPE B
R38	624	N	8	MANNHEIMIA
R39	578	N	8	MANNHEIMIA HAEMOLYTICA
R40	6536	N	13	PASTEURELLA
R41	44	N	3	PASTEURELLA HAEMOLYTICA
R42	1049	N	9	PASTEURELLA MULTOCIDA

?p

>>>Related terms display completed...

?logoff hold

245. The mutants were tested for growth yield, binding of F1 to membranes, dicyclohexylcarbodiimide sensitivity of ATPase activity, ATP-driven proton translocation, and passive proton permeability of membranes stripped of F1. Significant loss of function was seen only with insertions after positions 238 and 243. In contrast, both insertions after residue 225 and the alanine insertion after residue 245 were nearly identical in function to the wild type. The other insertions showed an intermediate loss of function. Missense mutations of His245 to serine and cysteine were nonfunctional, while the W241C mutant showed nearly normal ATPase function. Replacement of Leu162 by histidine failed to suppress the 245 mutants, but chemical rescue of H245S was partially successful using acetate. An interaction between Trp241 and His245 may be involved in gating a "half-channel" from the periplasmic surface of F0 to Asp61 of subunit a.

Tags: Comparative Study; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Histidine--genetics--GE; *Ion Channel Gating--genetics--GE; *Mutagenesis, Insertional; *Proton Pumps--genetics--GE; *Proton-Translocating ATPases--genetics--GE; Amino Acid Sequence; Amino Acid Substitution; Base Sequence; Escherichia coli; **Haemophilus influenzae**; Molecular Sequence Data; Protein Conformation; Proton-Translocating ATPases--metabolism--ME; Restriction Mapping; Structure-Activity Relationship; **Vibrio**

CAS Registry No.: 0 (Proton Pumps); 71-00-1 (Histidine)

Enzyme No.: EC 3.6.3.14 (Proton-Translocating ATPases)

Record Date Created: 19980803

Record Date Completed: 19980803

2/9/2 (Item 2 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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11177550 98053844 PMID: 9393695

In vitro Tn7 mutagenesis of **Haemophilus influenzae** Rd and characterization of the role of atpA in transformation.

Gwinn M L; Stellwagen A E; Craig N L; Tomb J F; Smith H O

Department of Molecular Biology and Genetics, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205, USA. mlgwinn@tigr.org

Journal of bacteriology (UNITED STATES) Dec 1997, 179 (23) p7315-20, ISSN 0021-9193 Journal Code: 2985120R

Contract/Grant No.: GM48251; GM; NIGMS; GM53824; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

Haemophilus influenzae Rd is a gram-negative bacterium capable of natural DNA transformation. The competent state occurs naturally in late exponential growth or can be induced by a nutritional downshift or by transient anaerobiosis. The genes cya, crp, topA, and sxy (tfoX) are known to function in the regulation of competence development. The phosphoenolpyruvate:carbohydrate phosphotransferase system functions to maintain levels of cyclic AMP necessary for competence development but is not directly involved in regulation. The exact signal(s) for competence and the genes that mediate the signal(s) are still unknown. In an effort to find additional regulatory genes, *H. influenzae* Rd was mutated by using an in vitro Tn7 system and screened for mutants with a reduced ability to induce the competence-regulatory gene, comA. Insertions in atpA, a gene coding for the alpha subunit of the F1 cytoplasmic domain of the ATP synthase, reduce transformation frequencies about 20-fold and cause a significant reduction in expression of competence-regulatory genes, while the expression of constitutive competence genes is only minimally affected. In addition, we found that an insertion in atpB, which encodes the a subunit of the F0 membrane-spanning domain, has a similar effect on transformation frequencies.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: **Haemophilus influenzae**--genetics--GE; *Proton-Translocating ATPases --genetics--GE; *Transformation, Genetic; Bacterial Proteins --genetics--GE; DNA Transposable Elements; DNA-Binding Proteins--genetics

--GE; Genes, Bacterial; *Haemophilus influenzae*--enzymology--EN; Ketone Oxidoreductases--genetics--GE; Mutagenesis, Insertional; Phenotype; Phosphoenolpyruvate Sugar Phosphotransferase System--genetics--GE; Phosphotransferases (Nitrogenous Group Acceptor)--genetics--GE; Selection (Genetics)

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA Transposable Elements); 0 (DNA-Binding Proteins); 0 (comA protein)
Enzyme No.: EC 1.2. (Ketone Oxidoreductases); EC 1.2.7.1 (pyruvate synthase); EC 2.7.1.- (Phosphoenolpyruvate Sugar Phosphotransferase System); EC 2.7.3 (Phosphotransferases (Nitrogenous Group Acceptor)); EC 2.7.3.9 (phosphoenolpyruvate-protein phosphotransferase); EC 3.6.3.14 (Proton-Translocating ATPases)

Record Date Created: 19971230

Record Date Completed: 19971230

2/9/3 (Item 3 from file: 155)

DIALOG(R)File 155: MEDLINE(R)

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10130168 22107276 PMID: 12112691

Crystal structure of the YjeE protein from *Haemophilus influenzae*: a putative Atpase involved in cell wall synthesis.

Telyakov Alexey; Obmolova Galina; Tordova Maria; Thanki Narmada; Bonander Nicklas; Eisenstein Edward; Howard Andrew J; Gilliland Gary L
Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute, Rockville, Maryland 20850, USA.
alexey@carb.nist.gov)

Proteins (United States) Aug 1 2002, 48 (2) p220-6, ISSN 1097-0134

Journal Code: 8700181

Contract/Grant No.: P01-GM57890; GM; NIGMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

A hypothetical protein encoded by the gene YjeE of *Haemophilus influenzae* was selected as part of a structural genomics project for X-ray analysis to assist with the functional assignment. The protein is considered essential to bacteria because the gene is present in virtually all bacterial genomes but not in those of archaea or eukaryotes. The amino acid sequence shows no homology to other proteins except for the presence of the Walker A motif G-X-X-X-G-K-T that indicates the possibility of a nucleotide-binding protein. The YjeE protein was cloned, expressed, and the crystal structure determined by the MAD method at 1.7-A resolution. The protein has a nucleotide-binding fold with a four-stranded parallel beta-sheet flanked by antiparallel beta-strands on each side. The topology of the beta-sheet is unique among P-loop proteins and has features of different families of enzymes. Crystallization of YjeE in the presence of ATP and Mg²⁺ resulted in the structure with ADP bound in the P-loop. The ATPase activity of YjeE was confirmed by kinetic measurements. The distribution of conserved residues suggests that the protein may work as a "molecular switch" triggered by ATP hydrolysis. The phylogenetic pattern of YjeE suggests its involvement in cell wall biosynthesis. Copyright 2002 Wiley-Liss, Inc.

Tags: Support, U.S. Gov't, Non-P.H.S.; Support, U.S. Gov't, P.H.S.

Descriptors: *Adenosinetriphosphatase--chemistry--CH; *Bacterial Proteins--chemistry--CH; **Haemophilus influenzae*--enzymology--EN; *Models, Molecular; Adenosinetriphosphatase--genetics--GE; Adenosinetriphosphatase--physiology--PH; Amino Acid Sequence; Bacterial Proteins--genetics--GE; Bacterial Proteins--physiology--PH; Cell Wall--metabolism--ME; Crystallography, X-Ray; *Haemophilus influenzae*--growth and development--GD; Molecular Sequence Data; Nucleotides--metabolism--ME; Phylogeny; Sequence Homology, Amino Acid

Molecular Sequence Databank No.: PDB/1FL9; PDB/1HTW

CAS Registry No.: 0 (Bacterial Proteins); 0 (Nucleotides)

Enzyme No.: EC 3.6.1.3 (Adenosinetriphosphatase); EC 3.6.1.3 (YjeE protein, *Haemophilus influenzae*)

Record Date Created: 20020711

Record Date Completed: 20020923

2/9/6 (Item 6 from file: 155)

DIALOG(R) File 155: MEDLINE(R)

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09671135 21461099 PMID: 11577153

Genetic diversity of *Pasteurella multocida* fowl cholera isolates as demonstrated by ribotyping and 16S rRNA and partial atpD sequence comparisons.

Petersen K D; Christensen H; Bisgaard M; Olsen J E

Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, 4 Stigboljen, DK-1870 Frederiksberg C, Copenhagen, Denmark. madsogkamille@mobilixnet.dk

Microbiology (Reading, England) (England) Oct 2001, 147 (Pt 10) p2739-48, ISSN 1350-0872 Journal Code: 9430468

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The genetic diversity of *Pasteurella multocida*, the aetiological agent of fowl cholera, was investigated. The strain collection comprised 69 clinical isolates representing a wide spectrum of hosts and geographic origin. The three type strains for the subspecies of *P. multocida* were also included. Avian isolates of *P. multocida* subsp. *multocida* and *P. multocida* subsp. *septica* did not represent separate lines by HpaII ribotyping and the two type strains of mammalian origin (porcine and cat bite) seemed to be representative of avian strains of *P. multocida* subsp. *multocida* and *septica*. By ribotyping, all *P. multocida* subsp. *gallicida* strains, except one chicken isolate and the type strain, clustered together. This indicated that the bovine type strain was not representative of this subspecies and that most strains of *P. multocida* subsp. *gallicida* are genetically related and may be distantly related to other *P. multocida* isolates, including those of avian origin. By 16S rRNA and atpD sequence comparisons of selected strains, including both *P. multocida* isolated from birds and mammals and selected distantly related *Pasteurella* species associated with birds and mammals, it was found that *P. multocida* is monophyletic. Extended DNA-DNA hybridizations are highly indicated since strains may exist which would connect the existing subspecies at species level. The considerable genetic diversity of *P. multocida* fowl cholera isolates is probably related to the clonal nature of this organism, resulting in many divergent lines.

Tags: Animal; Comparative Study; Support, Non-U.S. Gov't

Descriptors: *Bird Diseases--microbiology--MI; **Pasteurella* Infections --veterinary--VE; **Pasteurella multocida*--classification--CL; **Pasteurella multocida*--genetics--GE; *Variation (Genetics); Bacterial Proton-Translocating ATPases --genetics--GE; Birds; DNA, Ribosomal--analysis--AN; DNA, Ribosomal--genetics--GE; Evolution, Molecular; Genes, rRNA; Molecular Sequence Data; *Pasteurella* Infections--microbiology--MI; Phylogeny; RNA, Ribosomal, 16S--genetics--GE; Ribotyping; Sequence Analysis, DNA

Molecular Sequence Databank No.: GENBANK/AF326323; GENBANK/AF326324; GENBANK/AF326325

CAS Registry No.: 0 (DNA, Ribosomal); 0 (RNA, Ribosomal, 16S)

Enzyme No.: EC 3.6.1.- (Bacterial Proton-Translocating ATPases)

Record Date Created: 20010928

Record Date Completed: 20020314

?t s2/3,kwic/37 38

2/3,KWIC/37 (Item 24 from file: 349)

DIALOG(R) File 349:PCT FULLTEXT

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00428468 **Image available**

STREPTOCOCCUS PNEUMONIAE POLYNUCLEOTIDES AND SEQUENCES

POLYNUCLEOTIDES ET SEQUENCES DE STREPTOCOCCUS PNEUMONIAE

Patent Applicant/Assignee:

HUMAN GENOME SCIENCES INC,

KUNSCHE Charles A,

CHOI Gil H,

DILLON Patrick J,
ROSEN Craig A,
BARASH Steven C,
FANNON Michael,
DOUGHERTY Brian A,

Inventor(s):

KUNSCHE Charles A,
CHOI Gil H,
DILLON Patrick J,
ROSEN Craig A,
BARASH Steven C,
FANNON Michael,
DOUGHERTY Brian A,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9818931 A2 19980507
Application: WO 97US19588 19971030 (PCT/WO US9719588)
Priority Application: US 9629960 19961031

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES
FI GB GE GH HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK
MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN
YU ZW GH KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK
ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN
TD TG

Publication Language: English

Fulltext Word Count: 330745

Fulltext Availability:

Detailed Description

Detailed Description

... 1 74 1 60 | 462 | 137 1 9 1 6167 (6787 |gnl|PI0|dl00479 |Na+ -
ATPase subunit D [Enterococcus hirae] 1 74 ...157 1 2 1 243 | 824
|gi|1573373 |methylated-DNA--protein-cysteine methyltransferase (dat1) [
Haemophilus 1 74 1 48 | 582 |
1 1 1 1 influenzae] 1 1 1 1
164...74 1 50 | 1329 | 185 1 1 1 326 | 3 |gi|1573646 |Mg(2t)
transport ATPase protein C (mgtc) (SP:P22037) [Haemophilus 1 74 1
68 1 324 |
1 1 1 1 | influenzae] 1 1 I 1
188 1 2 1 1089 | 2018 |gi|1573008 (ATP dependent translocator homolog
(msBA) [Haemophilus influenzae] 1 74 1 44 | 930 | 189 111 1 6491
| 7174 |gi|1661199 | sakacin A...69 53 | 966 | 233 1 5 1 3249
1 4766 |gi|472918 |v-type Na- ATPase [Enterococcus hirae] | 69
56 (1518 | 235 1 3 1 660 1 1766 |gi|148945 (methylase [Haemophilus
influenzae] | 69 43 | 1107 | 243 1 2 1 865 1 2361
|gnl|PID|dl00225 IORFS...1 127 |12 1 | 7046 1 | 6606 1 |bhs| 153689 1
(HitB=iron utilization protein [Haemophilus influenzae, type b, DL42,
NTHI | j TN106, Peptide, 506 aaA(R) IHaemophilus influenzael
j 45 24 1 1 441 1 137 1 5 | 1561 | 2619 |gi|472921 |v-type Na- ATPase
(Enterococcus hirae) | 45 33 1 1059 1
209 1 1 | 774 | 364 |gi|304141 (restriction...

2/3,KWIC/38 (Item 25 from file: 349)

DIALOG(R)File 349:PCT FULLTEXT

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00350763

NUCLEOTIDE SEQUENCE OF THE HAEMOPHILUS INFLUENZAE Rd GENOME, FRAGMENTS
THEREOF, AND USES THEREOF
SEQUENCE NUCLEOTIDIQUE DU GENOME HAEMOPHILUS INFLUENZAE RD, DES FRAGMENTS
DE CE DERNIER, AINSI QUE SES APPLICATIONS

Patent Applicant/Assignee:

HUMAN GENOME SCIENCES INC,
JOHNS HOPKINS UNIVERSITY,

Inventor(s):

FLEISCHMANN Robert D,
ADAMS Mark D,
WHITE Owen,

SMITH Hamilton O,
VENTER J Craig,

Patent and Priority Information (Country, Number, Date):

Patent: WO 9633276 A1 19961024

Application: WO 96US5320 19960422 (PCT/WO US9605320)

Priority Application: US 95426787 19950421; US 95476102 19950607; US 95487429 19950607

Designated States: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN KE LS MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Publication Language: English

Fulltext Word Count: 154212

Fulltext Availability:

Detailed Description

Detailed Description

... system (kefC) (Escherichia coli)	40.9	65.7
594 H10292 326934 324769 potassium/copper-transportING	ATPase	
A (copA) (Enterococcus faecalis}	42.9	64.4
1428276 sodium/proline symporter...	723 H11355	1429787

...permease) (putP) (Escherichia coli)	62.8	79.1	489 H10252
283326 282517 tonB protein (tonB) (Haemophilus influenzae)			
96.2 98.5 261 H10627 664922 666362 TRK system potassium			
uptake protein (trkA...synthase (entC) (Bacillus subtilis) LA rn			
33.7 52.7 184 H11624 1686217 1685567membrane associated	ATPase	(cbiO)	
(Propionibacterium freudenreichii)			
34.2 56.0 221			
m tO H10463 481901 481029membrane protein (lapB) (Pasteurella			
haemolytica)			
63.1 80.2 216			
H11122 1184867 1185742membrane protein (lapB) (Pasteurella			
haemolytica)			
35.9 59.2 406			
H10590 608642 609874N-carbamyl-L-amino acid amidohydrolase (Bacillus			

...
?logoff hold

09671135 21461099 PMID: 11577153

Genetic diversity of *Pasteurella multocida* fowl cholera isolates as demonstrated by ribotyping and 16S rRNA and partial *atpD* sequence comparisons.

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The genetic diversity of *Pasteurella multocida*, the aetiological agent of fowl cholera, was investigated. The strain collection comprised 69 clinical isolates representing a wide spectrum of hosts and geographic origin. The three type strains for the subspecies of *P. multocida* were also included. Avian isolates of *P. multocida* subsp. *multocida* and *P. multocida* subsp. *septica* did not represent separate lines by *HpaII* ribotyping and the two type strains of mammalian origin (porcine and cat bite) seemed to be representative of avian strains of *P. multocida* subsp. *multocida* and *septica*. By ribotyping, all *P. multocida* subsp. *gallicida* strains, except one chicken isolate and the type strain, clustered together. This indicated that the bovine type strain was not representative of this subspecies and that most strains of *P. multocida* subsp. *gallicida* are genetically related and may be distantly related to other *P. multocida* isolates, including those of avian origin. By 16S rRNA and *atpD* sequence comparisons of selected strains, including both *P. multocida* isolated from birds and mammals and selected distantly related *Pasteurella* species associated with birds and mammals, it was found that *P. multocida* is monophyletic. Extended DNA-DNA hybridizations are highly indicated since strains may exist which would connect the existing subspecies at species level. The considerable genetic diversity of *P. multocida* fowl cholera isolates is probably related to the clonal nature of this organism, resulting in many divergent lines.

Tags: Animal; Comparative Study; Support, Non-U.S. Gov't

Descriptors: *Bird Diseases--microbiology--MI; **Pasteurella* Infections --veterinary--VE; **Pasteurella multocida*--classification--CL; **Pasteurella multocida*--genetics--GE; *Variation (Genetics); Bacterial Proton-Translocating ATPases --genetics--GE; Birds; DNA, Ribosomal--analysis--AN; DNA, Ribosomal--genetics--GE; Evolution, Molecular; Genes, rRNA; Molecular Sequence Data; *Pasteurella* Infections--microbiology--MI; Phylogeny; RNA, Ribosomal, 16S--genetics--GE; Ribotyping; Sequence Analysis, DNA

Molecular Sequence Databank No.: GENBANK/AF326323; GENBANK/AF326324; GENBANK/AF326325

CAS Registry No.: 0 (DNA, Ribosomal); 0 (RNA, Ribosomal, 16S)

Enzyme No.: EC 3.6.1.- (Bacterial Proton-Translocating ATPases)

Record Date Created: 20010928

Record Date Completed: 20020314

11177550 98053844 PMID: 9393695

In vitro Tn7 mutagenesis of *Haemophilus influenzae* Rd and characterization of the role of atpA in transformation.

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Haemophilus influenzae Rd is a gram-negative bacterium capable of natural DNA transformation. The competent state occurs naturally in late exponential growth or can be induced by a nutritional downshift or by transient anaerobiosis. The genes cya, crp, topA, and sxy (tfoX) are known to function in the regulation of competence development. The phosphoenolpyruvate:carbohydrate phosphotransferase system functions to maintain levels of cyclic AMP necessary for competence development but is not directly involved in regulation. The exact signal(s) for competence and the genes that mediate the signal(s) are still unknown. In an effort to find additional regulatory genes, *H. influenzae* Rd was mutated by using an in vitro Tn7 system and screened for mutants with a reduced ability to induce the competence-regulatory gene, comA. Insertions in atpA, a gene coding for the alpha subunit of the F1 cytoplasmic domain of the ATP synthase, reduce transformation frequencies about 20-fold and cause a significant reduction in expression of competence-regulatory genes, while the expression of constitutive competence genes is only minimally affected. In addition, we found that an insertion in atpB, which encodes the a subunit of the F0 membrane-spanning domain, has a similar effect on transformation frequencies.

Tags: Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Haemophilus influenzae*--genetics--GE; *Proton-Translocating ATPases --genetics--GE; *Transformation, Genetic; Bacterial Proteins --genetics--GE; DNA Transposable Elements; DNA-Binding Proteins--genetics --GE; Genes, Bacterial; *Haemophilus influenzae*--enzymology--EN; Ketone Oxidoreductases--genetics--GE; Mutagenesis, Insertional; Phenotype; Phosphoenolpyruvate Sugar Phosphotransferase System--genetics--GE; Phosphotransferases (Nitrogenous Group Acceptor)--genetics--GE; Selection (Genetics)

CAS Registry No.: 0 (Bacterial Proteins); 0 (DNA Transposable Elements); 0 (DNA-Binding Proteins); 0 (comA protein)

Enzyme No.: EC 1.2. (Ketone Oxidoreductases); EC 1.2.7.1 (pyruvate synthase); EC 2.7.1.- (Phosphoenolpyruvate Sugar Phosphotransferase System); EC 2.7.3 (Phosphotransferases (Nitrogenous Group Acceptor)); EC 2.7.3.9 (phosphoenolpyruvate-protein phosphotransferase); EC 3.6.3.14 (Proton-Translocating ATPases)

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11416069 98298134 PMID: 9632681

Insertion scanning mutagenesis of subunit a of the F1F0 ATP synthase near His245 and implications on gating of the proton channel.

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Subfile: INDEX MEDICUS

Subunit a of the *E. coli* F1F0 ATP synthase was probed by insertion scanning mutagenesis in a region between residues Glu219 and His245. A series of single amino acid insertions, of both alanine and aspartic acid, were constructed after the following residues: 225, 229, 233, 238, 243, and 245. The mutants were tested for growth yield, binding of F1 to membranes, dicyclohexylcarbodiimide sensitivity of ATPase activity, ATP-driven proton translocation, and passive proton permeability of membranes stripped of F1. Significant loss of function was seen only with insertions after positions 238 and 243. In contrast, both insertions after residue 225 and the alanine insertion after residue 245 were nearly identical in function to the wild type. The other insertions showed an intermediate loss of function. Missense mutations of His245 to serine and cysteine were nonfunctional, while the W241C mutant showed nearly normal ATPase function. Replacement of Leu162 by histidine failed to suppress the 245 mutants, but chemical rescue of H245S was partially successful using acetate. An interaction between Trp241 and His245 may be involved in gating a "half-channel" from the periplasmic surface of F0 to Asp61 of subunit a.

Tags: Comparative Study; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Histidine--genetics--GE; *Ion Channel Gating--genetics--GE; *Mutagenesis, Insertional; *Proton Pumps--genetics--GE; *Proton-Translocating ATPases--genetics--GE; Amino Acid Sequence; Amino Acid Substitution; Base Sequence; *Escherichia coli*; *Haemophilus influenzae*; Molecular Sequence Data; Protein Conformation; Proton-Translocating ATPases--metabolism--ME; Restriction Mapping; Structure-Activity Relationship; *Vibrio*

CAS Registry No.: 0 (Proton Pumps); 71-00-1 (Histidine)

Enzyme No.: EC 3.6.3.14 (Proton-Translocating ATPases)

Record Date Created: 19980803

Record Date Completed: 19980803